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CYTOLOGIC STUDIES ON RHEUMATIC FEVER

III. A COMPARISON OF CELLS OF SUBCUTANEOUS NODULES FROM PATIENTS WITH RHEUMATIC FEVER, RHEUMATOID ARTHRITIS AND SYPHILIS

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Although the subcutaneous nodules of rheumatic fever have received relatively greater attention than those of rheumatoid arthritis, the latter have been studied by a considerable number of investigators.[1] The clinical similarity between the nodules in the two diseases was early recognized and led to the suggestion[1a] that rheumatic fever and rheumatoid arthritis might be merely different manifestations of a single disease. More recently it has been shown[2] that the two types of lesions are even more strikingly similar in their histologic structure. An extensive and quite separate literature has accumulated, also, concerning the clinically very similar subcutaneous (juxto-articular) nodules encountered among patients with syphilis and those with yaws.[3]

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1. (*a*) Futcher, T. B.: Bull. Johns Hopkins Hosp. **6**:133, 1895. (*b*) Hawthorne, C. O.: Rheumatism, Rheumatoid Arthritis and Subcutaneous Nodules, London, J. & A. Churchill, 1900. (*c*) Wick, L.: Wien. med. Presse **45**:1117, 1173, 1234, 1280 and 1324, 1904; Wien. med. Wchnschr. **31**:1804, 1910. (*d*) Garrod, A. E., in Allbutt, T. C., and Rolleston, H. D.: A System of Medicine, New York, The Macmillan Company, 1910, vol. 3, p. 3. (*e*) Crouzon, O., and Bertrand, I.: Bull. et mém. Soc. méd. d. hôp. de Paris **50**:1401, 1926. (*f*) Dawson, M. H., and Boots, R. H.: J. A. M. A. **95**:1894, 1930.

2. (*a*) Coates, V., and Coombs, C. F.: Arch. Dis. Childhood **1**:183, 1928. (*b*) Freund, E.: Wien. Arch. f. inn. Med. **16**:73, 1928. (*c*) Coates, V.: Proc. Roy. Soc. Med. **23**:587, 1930. (*d*) Clawson, B. J., and Wetherby, M.: Am. J. Path. **8**:283, 1932. (*e*) Klinge, F., and Grzimek, N.: Virchows Arch. f. path. Anat. **284**:646, 1932. (*f*) Dawson, M. H.: J. Exper. Med. **57**:845, 1933. Dawson and Boots.[1f]

3. (*a*) Mayer, M., and Nauck, E. G.: Nodositas juxta-articularis, in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten, Berlin, Julius Springer, 1932. (*b*) Hoffman, H.: Juxtaarticulare Knoten, in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten, Berlin, Julius Springer, 1932. (*c*) Brunsting, L. A.: Am. J. Syph. **15**:42, 1931. (*d*) Hopkins, H. H.: Bull. Johns Hopkins Hosp. **49**:5, 1931.

In an earlier paper [4] a study of supravitally stained scrapings of rheumatic subcutaneous nodules was reported. The appearance of the characteristic cells of these lesions when stained with neutral red and Janus green was described, and it was concluded: (*a*) that these cells do not show with neutral red the reactions which distinguish monocytes, epithelioid cells and clasmatocytes, (*b*) that they probably arise from the undifferentiated mesenchymal cells of loose connective tissue and (*c*) that these observations probably apply also to the characteristic cells of rheumatic granulomas in the heart. Since the cardiac lesions do not lend themselves to study by supravital staining, the last conclusion was drawn purely from inference and was based on the clinical and pathologic evidence that rheumatic subcutaneous nodules are essentially the same as rheumatic granulomas elsewhere in the body. Some doubt has been cast on the validity of this assumption, however, by Hopkins' statement [3d] that the syphilitic nodules studied by him could not be differentiated microscopically from those associated with arthritis. This suggested the possibility that subcutaneous nodules might be merely nonspecific lesions occurring in various diseases and not representative of the characteristic pathologic changes of those diseases as found in other parts of the body. To test this possibility the present study was undertaken with the purpose of comparing supravitally stained preparations of subcutaneous nodules from patients with rheumatic fever, rheumatoid arthritis and syphilis.

MATERIAL AND TECHNIC

The tissues studied consisted of eleven rheumatic subcutaneous nodules previously described,[4] eight nodules from patients with rheumatoid arthritis,[5] two syphilitic juxto-articular nodules,[6] one gouty tophus and several sebaceous cysts, as well as normal human and rabbit subcutaneous tissues.

The rheumatic nodules were obtained from eleven children from 6 to 14 years of age, all of whom had typical rheumatic fever with characteristic cardiac involvement. None had clinical or serologic evidence of syphilis. The excised nodules had been present approximately from ten to ninety days prior to biopsy and measured from 0.25 to 0.5 cm. in diameter.

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4. McEwen, C.: J. Exper Med. **55**:745, 1932.

5. Five of the patients had been referred for biopsy and study from the clinic for patients with arthritis of the Presbyterian Hospital, New York, by Dr. R. H. Boots and Dr. M. H. Dawson.

6. The opportunity to study one of these patients was given me by Dr. J. P. Thornley and Dr. G. B. Rhodes, of the United States Marine Hospital 70, New York City.

Each of the eight patients with rheumatoid arthritis had chronic deforming polyarthritis typical of that disease. The youngest patient was 30 and the oldest 62 years of age. Four were females, and four, males. None had signs of heart disease, and none gave a history of having had rheumatic fever. There was no reason to suspect syphilis in any, and all gave negative Wassermann reactions. The earliest nodule in point of development had been present three weeks, and the oldest, two years. In size the lesions varied from 1 by 1 by 0.25 to 4 by 2 by 1 cm.; each excised lesion had been located at the characteristic site on the extensor surface of the forearm, just distal to the olecranon.

Both patients with syphilitic nodules were males. One was white and the other a Negro. Both were in the tertiary stage of syphilis; one had involvement of the central nervous system, while the other had no gross evidence of the disease other than the nodules. The Wassermann test was positive in both. In one the lesion had been present about six months and in the other about three years before the date of biopsy. In size and location these nodules were identical with those from patients with rheumatoid arthritis.

All nodules were excised under local anesthesia, care being taken to inject the procaine hydrochloride around and not into the substance of the nodule. Half of each excised nodule was placed in fixing solution for the preparation of ordinary microscopic sections; the other half, freed from extraneous tissue, was used for supravitally stained preparations. Since the technic was identical with that previously described,[4] it will be sufficient to say that scrapings of the nodules were exposed to weak dilutions of neutral red and Janus green on specially prepared slides and were examined at 37 C. with the oil immersion lens.

RESULTS

All supravitally stained preparations showed small masses of tissue composed of interwoven cells and fibrils. Most of these masses were too dense for a suitable study, but lying among them were large numbers of individual cells which could be satisfactorily examined.

Rheumatic Nodules.—The results of a study of supravitally stained scrapings of rheumatic nodules, described in detail elsewhere,[4] need only be summarized here. With the exception of a few lymphocytes, plasma cells, monocytes and clasmatocytes, the cells encountered appeared to be of one type; they varied considerably, however, in size and shape. The cell membrane was indistinct in contrast to the sharp outline of the nuclear membrane. The cytoplasm and nucleus had an almost identical coarse ground glass appearance, against which one or two nucleoli stood out clearly. Mitochondria were not seen. A striking feature was the failure of the cells to take up neutral red, only a few

EXPLANATION OF FIGURE 1

A, low power view of a part of a rheumatoid arthritic nodule showing large areas of collagenous necrosis surrounded by highly vascular and cellular granulation tissue. Hematoxylin and eosin; × 27.

B, higher magnification of the portion of field marked in *A*, showing pronounced new vessel formation and cellular proliferation. Hematoxylin and eosin; × 75.

C, cross-section of a syphilitic nodule showing a central cavity, a thick layer of relatively acellular fibrous tissue and the surrounding areolar tissue. The necrotic material which originally filled the central cavity has been lost. Hematoxylin and eosin; × 5.

D, higher magnification of the area marked in *C*. The paucity of cells and vessels in the fibrous tissue comprising the nodule and the collections of cells in the loose perinodular tissue are illustrated. Hematoxylin and eosin; × 27.

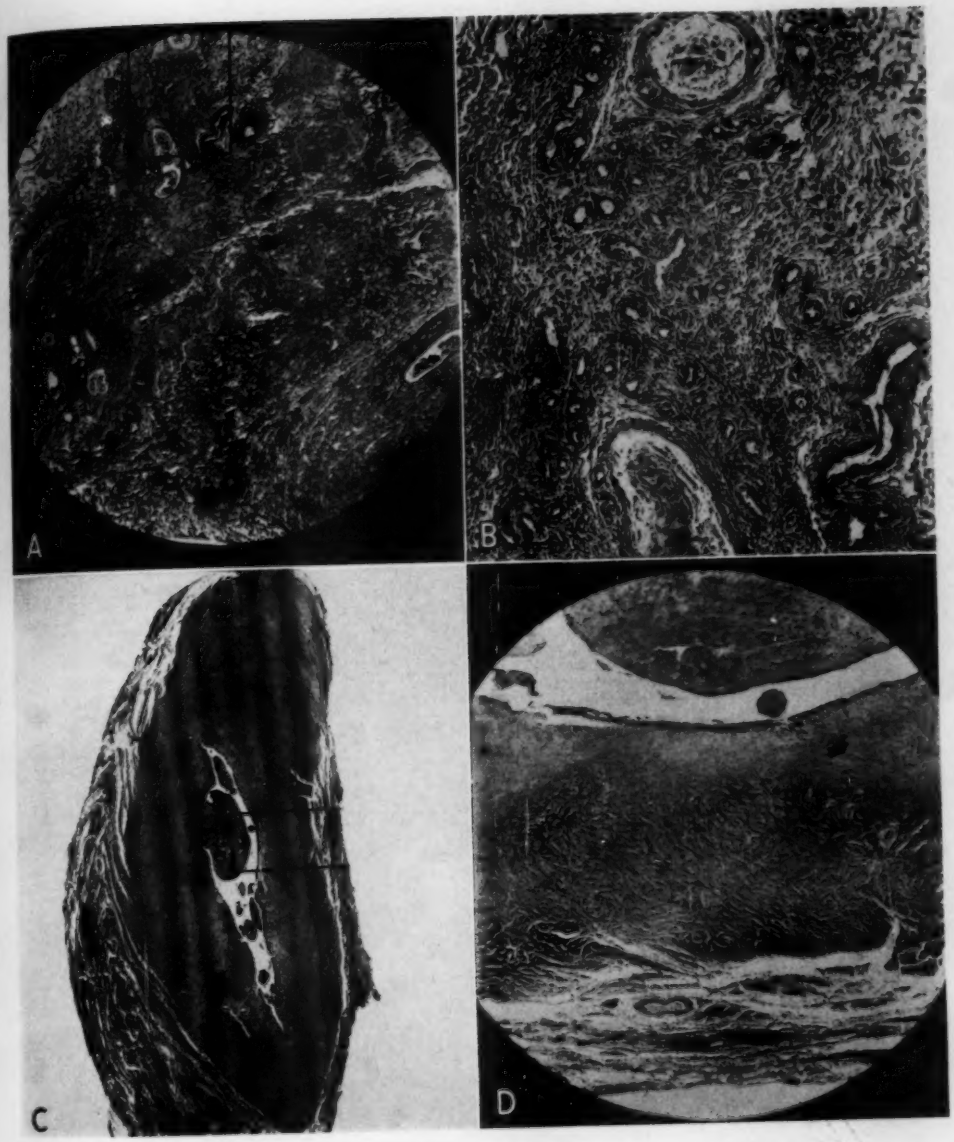

FIGURE 1

EXPLANATION OF FIGURE 2.

A, group of cells from a rheumatoid arthritic nodule, illustrating the sharp nuclear outline and nucleoli, the vague cytoplasmic detail and the failure to take up neutral red and Janus green. Nucleoli are beginning to stain from overexposure to neutral red. A crenate erythrocyte is included for comparison of size. Supravitally stained; × 1,000.

B, cell from a rheumatoid arthritic nodule, containing large refractive bodies. Supravitally stained; × 1,700.

C, cell from a rheumatoid arthritic nodule, representing transition toward the long spindle-shaped cells. Supravitally stained; × 1,700.

D, multinucleated cell from a syphilitic nodule. The many small bodies which appear white in the photograph were uniformly orange-red in the original. Supravitally stained; × 1,700.

E, large cell from a syphilitic nodule. The entire cytoplasm is filled with dark and light bodies, which obscure the nucleus and which in the original were various shades of red and orange. Parts of two air bubbles are included in the illustration. Supravitally stained; × 1,700.

F, cell from a syphilitic nodule, presenting some features of both monocytes and clasmatocytes. The neutral red bodies are uniformly dark red and of fairly uniform size. No mitochondria were seen. Supravitally stained; × 1,700.

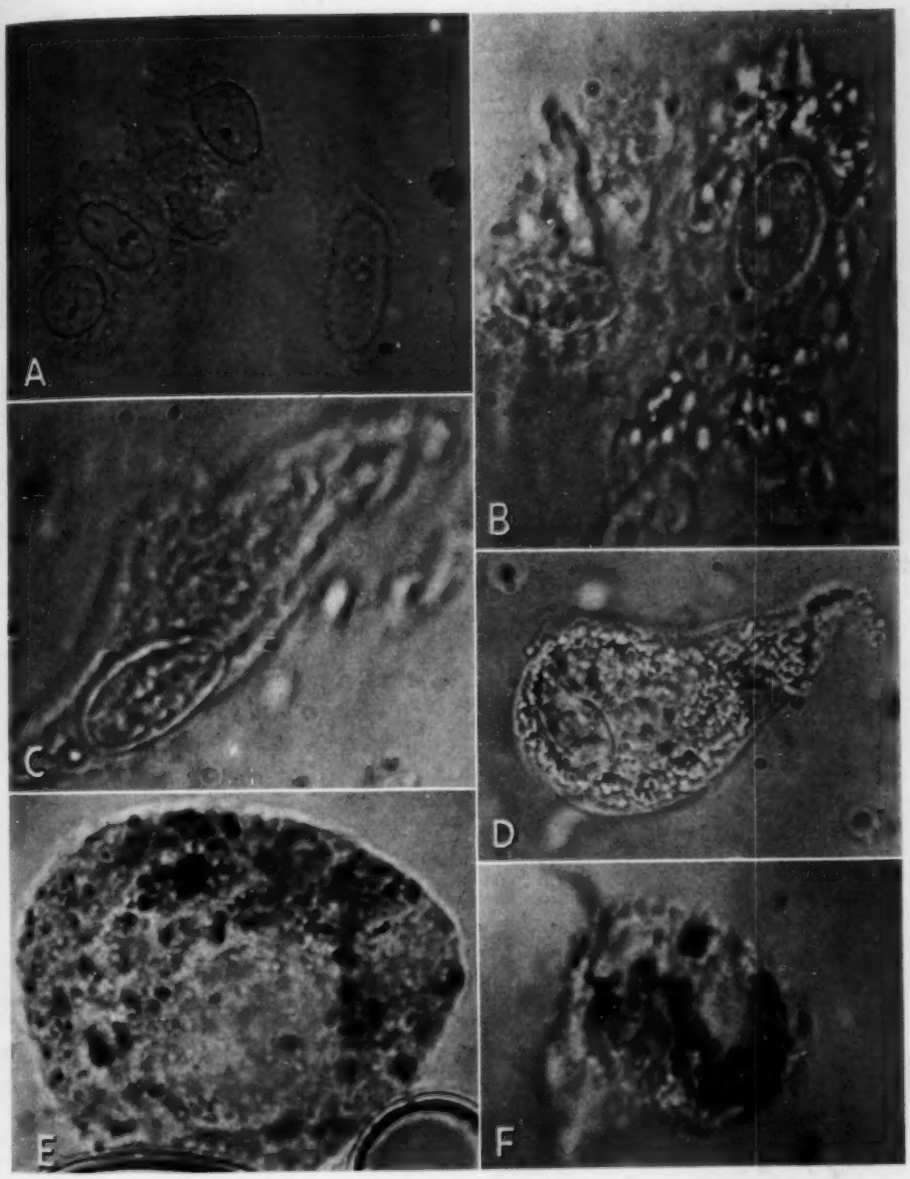

FIGURE 2

cells showing from 2 to 4 small pale pink dots, which faded after about thirty minutes. A few small refractive bodies were commonly present. The predominating cell was roughly oval and measured from 20 to 30 microns long and from 15 to 20 microns wide; however, others twice as large as this type were not infrequent. Cells with 2 or 3 nuclei were occasionally seen, and one with 11. All gradations between these oval cells and long spindle-shaped elements were noted. Cells having the general characteristics just described but no larger than intermediate lymphocytes were always present. These were thought to be primitive, undifferentiated connective tissue cells from which the others developed, or perhaps representative of an early stage of that differentiation. The cells of the predominant type were in all respects similar to those from a rheumatoid arthritic nodule, illustrated in figure 2 *A*. Actual cells from rheumatic nodules are not shown here since drawings and photomicrographs of the various forms have already been recorded in paper I of this series.[4]

Rheumatoid Arthritic Nodules.—The gross appearance of the nodules from the eight patients with rheumatoid arthritis of this series was identical with that described by Dawson and Boots.[1f] In histologic sections the similarities between nodules of rheumatic fever and rheumatoid arthritis, which have been repeatedly described (Wick [1c] and others [2]) were corroborated. Characteristic photomicrographs are shown in figures 1 *A* and *B*.

Examination of supravitally stained preparations of nodules from two patients with rheumatoid arthritis showed the cells to be both quantitatively and qualitatively identical with those of orthodox rheumatic nodules (fig. 2 *A*). The cells of the remaining nodules were essentially the same, but minor variations were noted. In five instances, from 0.5 to 10 per cent of the cells contained large refractive bodies such as are shown in figure 2 *B*, although usually smaller than those illustrated. These were never stained with neutral red but were glistening and tridimensional; possibly they represented a part of the process of degeneration. Another point of difference was the greater frequency of long spindle-shaped cells in the rheumatoid than in the rheumatic nodules; these were more than usually numerous in four of the nodules and in one comprised 30 per cent of the cells seen. Figure 2 *C* illustrates an intermediate form of spindle-shaped cell, having the characteristics of the typical cells shown in figure 2 *A* but definitely elongated and apparently developing in the direction of a fibrocyte. A third point to be noted was the presence of cells similar to the usual ones of rheumatic granulomas but containing small bodies which stained with neutral red. These were found in cells of only two nodules and as a rule were inconspicuous because of their minute size and pale color. They had the same appearance as the transiently staining bodies occasionally seen in

cells of true rheumatic lesions. In one nodule 15 per cent of the cells seen contained from 2 to 20 of these minute neutral red bodies. In no instance were the latter larger than 0.5 micron in diameter, and when a number occurred in a given cell all were of the same size and color, which varied from pale pink to orange and usually faded to yellow or was lost entirely by the end of one or two hours. Cells containing these bodies were easily distinguished from monocytes and clasmatocytes, which were noted in small numbers in one nodule. Occasionally, lymphocytes and what were considered to be plasma cells were seen. In scrapings from five of the eight nodules, multinucleated cells in every way identical with those of the rheumatic nodules were identified. There appeared to be no correlation between the age of the nodules in this small series and the relative frequency with which spindle-shaped elements or cells containing refractive or neutral red bodies were found.

Syphilitic Nodules.—The syphilitic nodules were strikingly different from the rheumatic and rheumatoid lesions. They consisted of firm white capsules from 1 to 4 mm. thick surrounding yellowish necrotic material. In one case the mass excised was made up of five smaller nodules closely bound together. Macroscopically these lesions appeared much less vascular than rheumatic and rheumatoid nodules. Microscopically they consisted of hyalinized connective tissue, which blended imperceptibly with the surrounding connective tissue fibers. At the center of each was a poorly defined cavity containing irregular hyalinized fragments possessing no definite structure. At the periphery of each nodule were collections of small round cells and larger cells containing brown pigment. The smaller blood vessels were moderately thickened but presented no characteristic features. The histologic structure distinguished these lesions from rheumatic and rheumatoid nodules, as has been pointed out by Crouzon and Bertrand [1e] and Dawson.[2f] Photomicrographs of one nodule are shown in figure 1 *B* and *C*.

The findings in supravitally stained preparations also were strikingly different from those of rheumatic and rheumatoid nodules and were in general agreement with observations made on experimentally induced syphilitic lesions in rabbits. Morgan and Cunningham and their associates [7] studied supravitally stained scrapings of experimentally induced syphilitic lesions and found the predominant cells to be highly phagocytic large macrophages (clasmatocytes) containing many vacuoles of varying size and color. In similar studies Pearce and Rosahn,[8] on the other

7. (*a*) Morgan, H. J.: Tr. A. Am. Physicians **45**:67, 1930. (*b*) Cunningham, R. S.; Morgan, H. J.; Tompkins, E. H., and Harris, S., Jr.: Am. J. Syph. **17**: 515, 1933.

8. Pearce, L., and Rosahn, P. D.: Proc. Soc. Exper. Biol. & Med. **28**:654, 1931.

hand, found normal and stimulated monocytes in early lesions and clasmatocytes only later. In the two nodules which I examined, almost every cell took up neutral red in greater or less amount, and all cell types reported in experimental lesions were encountered. Normal, unstimulated monocytes were present in small numbers, but the predominant cells were of two types: (*a*) typical highly phagocytic clasmatocytes containing many vacuoles which varied in size and color (fig 2 *E*) and (*b*) cells containing smaller vacuoles of uniform size and color (fig. 2 *F*). The color in the latter, although uniform within a given cell, varied from yellow to deep red in different cells, the predominant shade being a dark salmon. In some of these cells, as in the one illustrated, only a moderate number of these neutral red bodies were seen, while in others they were so numerous that the nuclei were obscured; the cell membrane, in contrast, was always sharply outlined. One cell with multiple nuclei was noted (fig. 2 *D*). Whether these cells should be classified as clasmatocytes or as peculiar stimulated monocytes is uncertain, but they appear to be fairly characteristic of syphilitic lesions, as they occurred relatively frequently in the two human nodules described here, as well as in rabbit lesions placed at my disposal by Dr. Louise Pearce and Dr. P. D. Rosahn.

In the various preparations from the two human nodules a few small cells such as those found in rheumatic and rheumatoid granulomas were observed, but none of the characteristic large oval cells of the type predominating in the latter leisons, and no free spindle-shaped forms, although the latter were identified in the small tissue masses always present.

Controls.—Supravitally stained preparations of other subcutaneous lesions and tissues were examined as controls. These included a gouty tophus, a number of sebaceous cysts and normal human and rabbit subcutaneous tissue, fascia and tendons. In the study of the tophus and sebaceous cysts, care was taken to scrape only the fibrous capsules. The cells found in these scrapings were strikingly few compared with those from the granulomas. The preparations made from the tophus revealed merely a few spindle-shaped elements and small oval cells of the type noted in rheumatic and rheumatoid nodules and thought to be primitive, undifferentiated connective tissue cells. A very few of these small cells were found also in the scrapings of sebaceous cysts, but the chief cells present in the latter were clasmatocytes, basophils and lymphocytes. The normal tissues in supravitally stained preparations revealed merely a few spindle-shaped and small oval cells and large masses of apparently acellular fibrils. None of the characteristic large mononuclear or multinuclear cells of rheumatic and rheumatoid nodules was seen in any control tissue.

COMMENT

In this study, the predominant cells of syphilitic subcutaneous nodules differed strikingly from those of rheumatic and rheumatoid arthritic nodules in their reaction to supravital staining and proved to be the distinctive stimulated monocytes and clasmatocytes previously shown [9] to be characteristic of syphilitic lesions. Thus, cytologic proof is added to the evidence provided by ordinary histologic study that the clinically similar subcutaneous nodules of rheumatoid arthritis and syphilis are pathologically dissimilar; the latter are shown to be not merely nonspecific lesions but representative of syphilitic tissue reactions in general. This result gives indirect support to the belief that in rheumatic fever, too, the subcutaneous nodules are characteristic of granulomas elsewhere in the body and that conclusions drawn from a study of cells of the nodules are applicable also to those of the cardiac lesions.[4]

In contrast to the findings in the syphilitic lesions, the cells of nodules from patients with rheumatoid arthritis were in all essential features the some as those of orthodox rheumatic nodules. Obviously, this does not prove the identity of rheumatic fever and rheumatoid arthritis, but it does add one more bit of evidence to the clinical and histologic similarities suggesting a relationship between these two diseases, and, when taken into consideration with other histologic features, it indicates that at least in the proliferative phase of the tissue reaction they are similar.

Study of control material revealed that scrapings of normal connective tissues contained small numbers of the spindle-shaped cells and small oval cells noted in rheumatic granulomas. Probably the former are mature fibrocytes and the latter primitive, undifferentiated connective tissue cells, possessing, however, great capacity for differentiation in various functional directions when suitably stimulated. These small cells were more abundant in the zone of connective tissue reaction surrounding cysts and tophi, but although increased in numbers they failed to show differentiation into the large and sometimes multinucleated cells of rheumatic and rheumatoid granulomas. In a previous paper,[4] it was reported that scrapings of very early lesions of experimentally induced tuberculosis contain the same oval cells in abundance and, in addition, a moderate number of the larger cells noted in rheumatic granulomas; soon, however, these cells are converted into the monocytes and epithelioid cells characteristic of tuberculosis. In rheumatic fever and rheumatoid arthritis, on the other hand, although the young connective tissue cells undergo marked increase in number and size and tend to develop into multinucleated forms, they show little or

9. Morgan.[7a] Cunningham and others.[7b] Pearce and Rosahn.[8]

no differentiation so far as their capacity to react to neutral red and Janus green is concerned.

SUMMARY

A comparative study is reported of supravitally stained preparations of subcutaneous nodules from patients with rheumatic fever, rheumatoid arthritis and syphilis. The cells of rheumatoid arthritic nodules were found to have essentially the same characteristics as those of rheumatic fever. The cells of syphilitic nodules differed and proved to be those characteristic of syphilitic lesions in general. The bearing of these results on the nature of the characteristic cells of rheumatic granulomas in the heart and on the relationship between rheumatic fever and rheumatoid arthritis is discussed.

BENZENE POISONING WITH BIZARRE EXTRAMEDULLARY HEMATOPOIESIS

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Accumulated evidence has directed attention to the close relation between the various active and potentially active components of the hematopoietic system. Knowledge concerning this closely knit system has grown out of observations of unsuspected reactions to supposedly well known stimuli. It is not amiss, therefore, that the following histologically peculiar case of benzene poisoning should be presented.

Among the varied potentialities of the reticulo-endothelial system is the apparent ability to resume fetal extramedullary hematopoiesis, a function which is dormant in the adult state. The relative frequency with which the liver and spleen exhibit such activities under various clinical and experimental conditions is generally recognized. These histologic changes are, however, distinctly rare in clinical aplastic anemia resultant on benzene poisoning.

Benzene is known to have a markedly destructive action on the bone marrow and, varying with the individual susceptibility and dosage, aplasia may ensue to a point incompatible with life (Hamilton;[1] Hunter and Hanflig;[2] Selling[3]). Resultant on the destructive process there is produced in human beings progressive leukopenia, anemia and thrombocytopenia. Under such extreme circumstances as these myeloid metaplasia of extramedullary organs is prone to occur, and it is surprising that changes of this nature are not more frequently reported in benzene poisoning.

Many persons subjected to benzene fumes, although exhibiting profound changes in the peripheral blood and a course marked by hemorrhagic phenomena, eventually progress to seemingly complete recovery. Continued studies of the blood, however, have always shown some residual evidence of abnormality. Selling,[3b] Hamilton[1] and others have made note of this point, and it is perfectly conceivable that the damage

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1. Hamilton, A.: Arch. Path. **11**:434 and 601, 1931.

2. Hunter, F. T., and Hanflig, S. S.: Boston M. & S. J. **197**:292, 1927.

3. Selling, L.: (*a*) Bull. Johns Hopkins Hosp. **21**:33, 1910; (*b*) Johns Hopkins Hosp. Rep. **17**:83, 1916.

of which benzene is capable might have under certain circumstances a permanent although nonlethal effect. That this effect may be stimulatory as well as inhibitory has been shown to be a distinct possibility (Andersen;[4] Medlar;[5] Selling[3b]). The case herein reported is in part confirmatory and in addition presents several other points of striking interest.

HISTORY OF CASE

A 45 year old American leather worker was admitted to the Baker Memorial, under the care of Dr. Francis T. Hunter. He complained of weakness and pains in the legs.

Six months prior to entry he noted gradually progressive shortness of breath, dizziness and sharp cramplike pains in his extremities. After a short time he observed gradual development of pallor and weakness, which became sufficiently advanced two months before he came to the hospital to cause him to cease working. There had been frequent frontal headaches, irritability and insomnia. For an unstated period he had taken large amounts of liver and iron for the anemia. Weakness and disability were, however, progressive, and on occasion small purpuric spots appeared on the skin of the extremities, although early in the disease hemorrhagic manifestations were minimal.

The patient's occupation had required his exposure to benzene, ethyl acetate and butanol for about four years. One year before entry one of his co-workers who had been particularly exposed to these substances died of anemia. The patient took over his comrade's work and six months later became ill in turn. His past history was otherwise noncontributory.

Physical examination showed a moderately obese middle-aged white man in no acute distress. The skin and mucous membranes were extremely pallid and the nail beds almost white. On the palate and cheeks and along the gingival margins were many poorly defined brown-blue pigmented areas. The retinas exhibited several fresh hemorrhages of irregular form, and there were a few small yellowish patches of old exudate in the right fundus. The heart was not enlarged, but soft systolic murmurs were heard in the apical and aortic areas. The blood pressure was 120 systolic and 50 diastolic. The lungs were clear. The abdomen was large and soft, and although neither liver nor spleen was palpable the area of splenic dulness was increased. Over both shins were areas of brownish pigmentation, suggestive of fading ecchymoses. The tourniquet showed definitely decreased capillary resistance.

Examination of several urine specimens disclosed nothing noteworthy. Three stool specimens obtained during the first week in the hospital gave negative reactions to the guaiac test. The reaction to the Hinton test was negative. The icterus index was 5.

An x-ray film of the chest was essentially normal.

At entry the red blood cell count was 1,200,000 per cubic millimeter, with 40 per cent hemoglobin (Sahli). The white cells numbered 2,400 per cubic millimeter, with 24 per cent polymorphonuclear neutrophils, 53 per cent lymphocytes, 11 per cent monocytes, 2 per cent basophils, 4 per cent unclassified cells and 6 per cent nucleated red blood cells. Reticulocytes numbered 3.8 per cent. There

4. Andersen, D. H.: Am. J. Path. **10**:101, 1934.

5. Medlar, E. M.: Folia haemat. **53**:397, 1935.

was an occasional polychromatophilic cell, and platelets were rare. The bleeding time was five and a half minutes. Daily studies of the blood were made, but for the sake of brevity only weekly results are tabulated (table).

Fluctuations in the number of erythrocytes occurred frequently but could generally be accounted for by the fact that the patient received fourteen transfusions during his stay in the hospital. Polychromatophilia was evident at all examinations, and there were marked anisocytosis and poikilocytosis, with a fairly large number of macrocytes. Most of the erythrocytes appeared to be well filled with hemoglobin. Occasional stippled cells were seen, but the basophilic substance was always coarsely granular in appearance. Platelets were consistently few, and the bleeding time remained elevated, ranging between five and thirty-five minutes.

Blood Picture at Weekly Intervals in Benzene Poisoning

Hospital Day	Red Blood Cells	Hemoglobin, Percentage	White Blood Cells	Polymorphonuclears	Lymphocytes	Monocytes	Eosinophils	Basophils	Myelocytes	Myeloblasts	Unclassified	Nucleated Red Blood Cells	Reticulocytes
1	1,200,000	40	2,400	24	53	11	..	2	..	..	4	6	3.8
**													
8	2,500,000	50	2,200	20	56	7	3	2	2	..	9	1	2.4
*													
16	3,300,000	58	4,700	21	58	15	..	2	3	..	..	1	1.4
**													
22	3,800,000	56	3,300	37	44	15	1	..	..	..	2	1	1.0
*													
29	3,500,000	57	1,300	17	47	15	1	..	..	2	15	3	...
*													
36	3,500,000	65	2,400	14	63	19	..	2	..	..	2	..	...
***													
43	3,100,000	60	2,700	17	74	6	1	1	..	..	1	..	...
50	1,100,000	38	4,100	10	65	1	..	1	1	1	20	1	...
***	A biopsy of the bone marrow was made												
57	2,100,000	33	1,300	29	50	9	..	..	..	3	9	..	...
*													
61	1,000,000	24	3,500	16	54	12	..	..	2	..	12	4	...

* Asterisks signify the number of transfusions administered during the intervals between the hospital days noted.

Throughout the period of hospitalization the patient's temperature exhibited daily variations between 98.6 and 101 F. with occasional rises to 103 and 105. In addition to the transfusions the patient received ferrous sulfate, liver extract parenterally and pentnucleotide. None of these substances produced any significant change in the blood constituents, which were influenced only by transfusions and then transiently. The patient complained frequently of severe pains in the extremities, but neurologic examinations gave entirely negative results. At the end of a month and a half of hospitalization epistaxis occurred and subsequently became frequent and annoying. On the fifty-second hospital day a sternal biopsy was made. Bleeding from the wound was sufficiently stubborn to require muscle packing to secure hemostasis.

On the sixty-first hospital day there was emesis of a large amount of blood. The patient's condition became rapidly worse, and he died two days later, two months after entry and eight months after the onset of symptoms.

Imprint preparations made from the marrow taken for biopsy and stained with Giemsa stain showed nucleated cells of the erythroid series in seeming abundance. The fixed section, however, exhibited extensive replacement of the marrow by loose fibrillar tissue with vesicular fibroblastic nuclei. Within the stroma lay blood elements in markedly diminished numbers. Granulocytes were evident for the most part as immature forms, but their numbers were considerably less than normal, and they were arranged in sparsely cellular groups. Of the mature leukocytes, eosinophilic cells predominated. Red cell progenitors comprised the largest number of cells present, and they too appeared in clumps and clusters, generally lying within endothelial-lined spaces. Normoblasts and erythroblasts were readily discerned in moderate numbers, but cells identified in the imprint preparations as megaloblasts were more abundant. Megakaryocytes were absent.

There can be little question regarding the clinical diagnosis in this case. The patient had had prolonged contact with benzene vapor and had as a development therefrom profound anemia, granulopenia and thrombocytopenia. There were associated hemorrhagic phenomena, and biopsy of marrow showed extensive fibrous replacement with diminution in erythropoiesis, which was obviously insufficient to carry on adequate production of blood. The terminally combined loss of blood and relative aplasia of the marrow served to produce death. Up to this point no unusual features have been considered, and the findings are those universally recorded in clinical textbooks.

There were, however, several clinical observations relatively unusual in this disease. Closely allied to the deficiency in the marrow and the qualitative character of the regenerating islands were the low grade reticulocytosis and macrocytosis. Probably nucleated red cells occurred in the peripheral blood for similar reasons. In the Cabot case [6] there were 6 per cent reticulocytes and in Andersen's [4] 12 per cent. Castle and Minot [7] and Hamilton [1] have commented on the occasional occurrence of macrocytosis in benzene poisoning. Selling [3] and Bunting [8] observed nucleated red cells in the circulating blood in aplastic anemia.

Myelocytes and myeloblasts in the blood smears were undoubtedly resultant on extrusion of immature cells from hematopoietic centers that were incapable of replenishing peripheral blood needs in a normal fashion. Study of the so-called unclassified cells in this case showed that many of them belonged in the myeloid group, the remainder being atypical monocytes, lymphocytes and plasma cells. Hunter and Hanflig [2] stated that four of their five cases exhibited circulating myelocytes or less mature forms, and similar observations were made in seventeen of

6. Bleeding from the Gums, Cabot Case 13321, Boston M. & S. J. **197**:236, 1927.

7. Castle, W. R., and Minot, G. R.: Pathological Physiology and Clinical Description of the Anemias, New York, Oxford University Press, 1936, p. 91.

8. Bunting, C. H.: J. Exper. Med. **8**:625, 1906.

the thirty-two cases in Smith's [9] series. A relative increase in circulating monocytes like that in half of Smith's cases was noted in this case.

The histologic features of the bone marrow will be considered in detail in conjunction with the observations at necropsy. It suffices to state at this time that the appearance of the marrow was altogether consistent with early regeneration of a previously scarred marrow.

Necropsy (The following observations were made available by Dr. William J. Brickley, medical examiner for Suffolk County, Northern Division).—The body was that of a well developed, moderately obese 45 year old white man, measuring 5 feet and 10 inches (178 cm.) and weighing 165 pounds (75 Kg.). There was pronounced pallor of the skin and visible mucous membrane. Scattered over the torso and extremities were numerous purpuric and ecchymotic areas measuring from 1 mm. to 6 cm. in diameter. Overlying the sternum at the level of the third rib was an unhealed, gaping surgical wound measuring 2.5 cm. in length. The subcutaneous fat was abundant, and the muscles were normal in appearance.

The peritoneal cavity showed nothing abnormal.

The stomach contained 1,000 cc. of brownish black fluid, within which were numerous dark brown shreds. The mucosa exhibited an irregular red-brown hemorrhagic mottling but was otherwise normal. The entire intestinal tract was filled with brownish black and tarry liquid stool. No bleeding point was identified. The mesenteric and retroperitoneal lymph nodes were normal.

The pleura contained a few minute hemorrhagic spots measuring up to 2 mm.

The heart weighed 350 Gm. and except for a few epicardial hemorrhagic spots was without gross change. The coronary arteries contained several scattered non-calcified atheromatous plaques.

The liver weighed 2,000 Gm. The surface was pale yellowish brown, and the edge was sharp. On section the parenchyma was pallid and rather soft in consistency. The lobules exhibited moderately increased prominence, but the central veins were not evident.

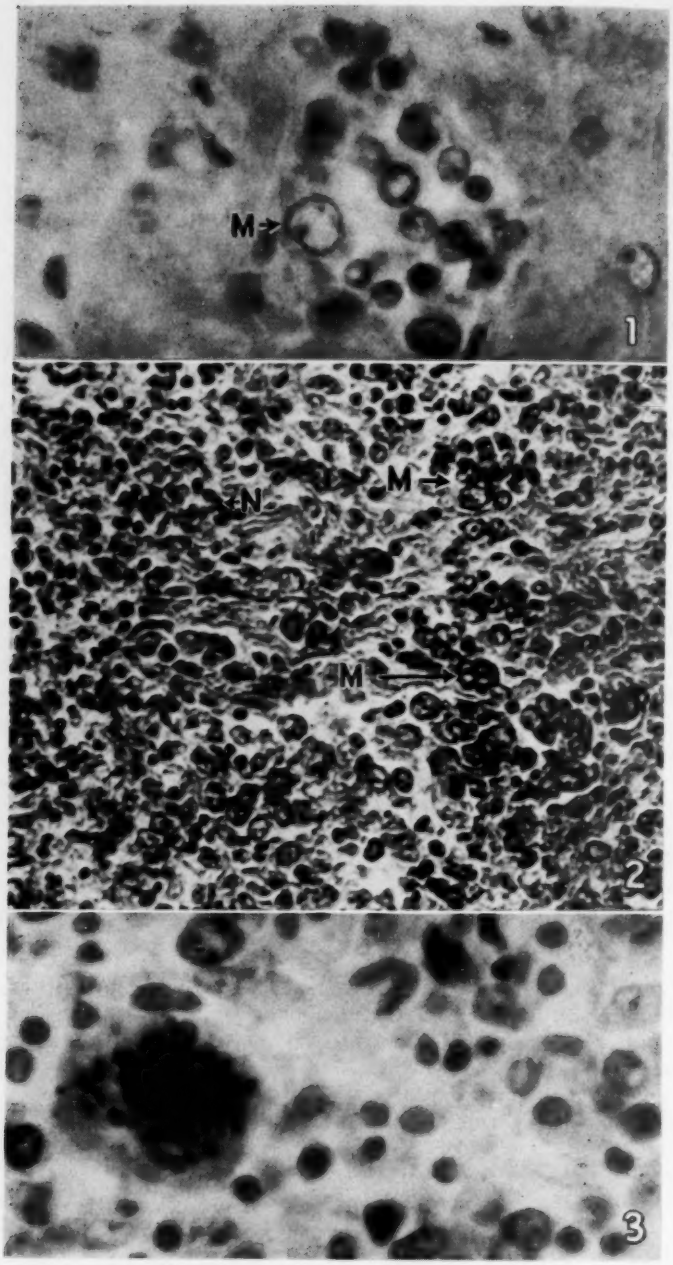
The spleen weighed 320 Gm. Its surface was smooth and bluish gray, with scattered, poorly circumscribed mottled reddish areas measuring up to 2.5 cm. in diameter. On section the parenchyma was grayish brown and contained many poorly defined hemorrhagic areas measuring up to 2 cm. Normal landmarks were otherwise evident.

The bone marrow of the vertebrae and sternum was pale grayish red, and bony trabeculation was grossly increased. The marrow removed from the upper third of the femur was reddish brown and gelatinous.

All other organs examined exhibited pronounced pallor but were otherwise grossly normal in appearance.

Microscopic Examination.—The liver showed focal areas of central necrosis, within which few polymorphonuclear leukocytes were seen. The exudative cells in these regions were mainly monocytes, scattered lymphocytes and, in moderate numbers, cells similar to those noted within the sinusoids. Scattered sparsely throughout the sinusoids, singly and in small clusters, were peculiar cells. These occasionally appeared to be attached to and budding from the unusually prominent lining endothelial cells. For the most part they lay free in the lumens, and occasional sinusoids were dilated to contain nests of from 15 to 20 of them (fig. 1). The cells measured from 12 to 15 microns in diameter and possessed

9. Smith, A. R.: J. Indust. Hyg. & Toxicol. **10**:73, 1928.



EXPLANATION OF FIGURES 1, 2 AND 3.

Fig.1.—A high power view of a section of liver demonstrating a sinusoid distended by erythrogenic cells. Note the large megaloblast (*M*) adjacent to the wall of the sinusoid. The remainder of the cells are predominantly erythroblasts, with a few normoblasts. Poor fixation precluded sharp distinction of the parenchymatous cells, which exhibit a smudgy appearance.

Fig. 2.—A low power view of spleen exhibiting very marked intrasinusoidal cellularity. Practically all of the cells are erythrogenic. The larger cells with clear vesicular nuclei and scanty dark staining cytoplasm are megaloblasts (*M*). Normoblasts (*N*), with dark pyknotic nuclei, may be readily identified.

Fig. 3.—A high power view of spleen showing a large cell containing a multipolar mitotic figure lying within a sinusoid. The other cells are similar to those in figure 4.

large oval and round vesicular nuclei, centrally placed, with broad slightly refractile nuclear membranes. The cytoplasm was comparatively scanty, slightly basophilic and agranular. A few of the nuclei contained single nucleoli. A rare multinucleated giant cell was observed. There was no hemosiderosis.

The spleen was the seat of an extraordinary degree of hematopoiesis. The lymphoid follicles were diminished in number, and the central arterioles exhibited moderate intimal hyaline thickening. Billroth's cords were narrow and formed an insignificant portion of the picture. The striking feature was the very marked intrasinusoidal cellularity. Littoral cells were swollen and projected into the sinusoids. Attached to the walls and lying free in the lumens were large cells similar to those described in the liver, in enormous numbers (fig. 2). Many of these, however, measured up to 25 microns, and their nuclei were paler and more swollen and contained smudgy clumps of chromatin. Mitotic figures were numerous, and a few cells contained two and three discrete nuclei. Nuclear borders were thinner, and the cytoplasm was slightly more abundant and somewhat more basophilic. On the basis of comparison with identical cells in the bone marrow recognized by their characteristics in imprint preparations, these were identified as megaloblasts.

From the large megaloblasts there occurred evident though scanty differentiation into erythroblasts and normoblasts. Maturation was apparently greatly impeded. In rare scattered areas myelocytes were discerned, but development was essentially erythroid. Oxydase stains applied to both liver and spleen showed the large cells to be oxydase negative. The less numerous granulocytes contained stainable oxydase granules.

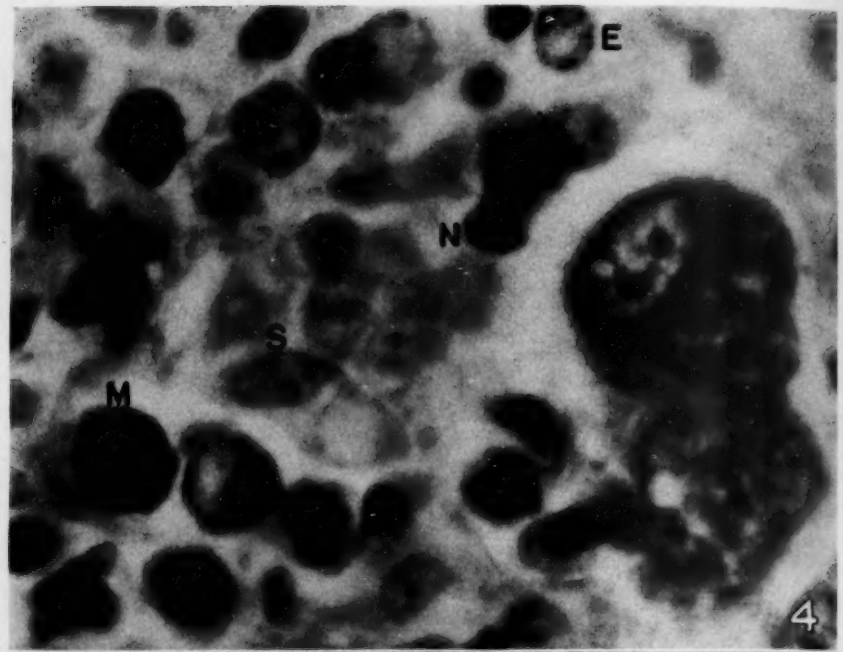
Accompanying the hematopoietic cells were large numbers of phagocytic monocytes and clasmatocytes containing an abundance of hemosiderin. Other cells, evidently megakaryocytes, contained several large superimposed nuclei (fig. 4) and occasionally multipolar mitoses. The appearance of these mitotic figures was impressive (fig. 3).

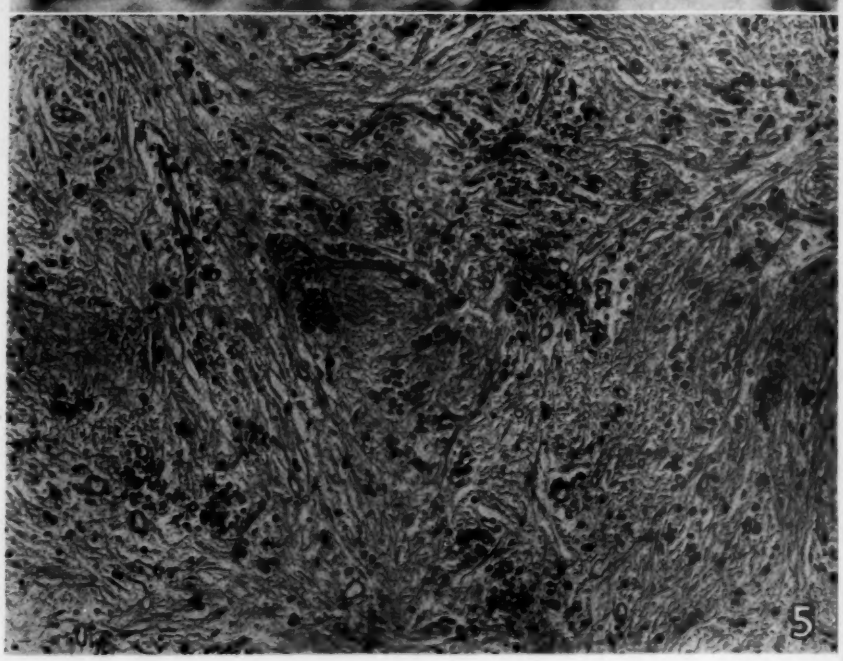
The architecture of the bone marrow wherever examined was obscured for the most part by a loose, rather fibrillar connective tissue stroma containing oval and fusiform fibroblastic nuclei, pale staining and active looking. In occasional focal areas these were irregularly aggregated, and the fibrils assumed a rather bizarre whorled arrangement (fig. 5). Abundantly scattered throughout were small clusters of hemosiderin granules, and there were large numbers of macrophages loaded with similar material. In comparatively increasing, but still relatively scanty amounts, hematopoiesis was evident in the sternum, femur and vertebrae. In the latter an occasional nodular area of intense hyperplasia was noted. As elsewhere, erythropoiesis was predominant, and this was fairly definitely intrasinusoidal. In these areas megaloblasts similar to those noted in the splenic sinusoids, erythroblasts and normoblasts frequently lay closely aggregated in fairly large clusters. There was extreme paucity of leukopoiesis. Few mature polymorphonuclear leukocytes were observed, and of these eosinophils were in the majority. Megakaryocytes were few, pale, swollen and, in contradistinction to those in the spleen, rather effete in appearance. At no portion of the marrow was cellular activity or the appearance of immaturity as marked as it was in the spleen.

The lungs were essentially normal, but an occasional small artery contained a thrombus, within the fibrinous meshwork of which were numerous cells similar to those noted in the spleen and liver.

Microscopic examination of other organs gave essentially negative results.

Cultures of blood taken from the heart at autopsy showed a scant growth of Staphylococcus aureus.





EXPLANATION OF FIGURES 4 AND 5.

Fig. 4.—A very high power view of spleen showing a large multinucleated megakaryocyte within a sinusoid. Note the large numbers of megaloblasts (*M*), erythroblasts (*E*), normoblasts (*N*) and splenic stromal cells (*S*). No granulocytic elements are seen.

Fig. 5.—A low power view of vertebral marrow demonstrating marked fibrous replacement of marrow substance. Note the irregular whorl-like arrangement of the stroma and the focal islands of regenerative hematopoiesis.

The relatively youthful and active appearance of the fibroblastic nuclei in a few areas of the marrow was sufficiently striking to recall the "radiation osteitis" described by Martland in cases of radium poisoning.[10] Likewise, the focal hematopoietic hyperplasia of the marrow in this case and the diffuse hyperplasia occurring in other reported cases of benzene poisoning simulated the earlier phases of "radiation osteitis."

The free clusters and phagocytosed particles of hemosiderin granules were of interest in view of the fact that Piney[11] had claimed that hemosiderosis never occurs in benzene poisoning. It has, however, been observed by others in many cases (Hamilton;[1] Andersen;[4] Smith;[9] the Cabot case[6]); in fact, Long[12] noted the presence of hemosiderin-laden monocytes in the circulating blood in experimentally produced aplastic anemia. It is probable that the peripheral monocytosis recorded by Smith[9] and noted in the present case is closely related to the marrow requirement for phagocytic cells in the presence of marked parenchymatous destruction.

Hemosiderosis is generally significant of active erythrolysis. In view of the fact that contact with benzene had ceased in this case at least four months before death, it is puzzling that hemosiderosis should be so prominent. One may consider three possible explanations for this. It is conceivable that benzene or its by-products may be retained and cause continued destruction of the mature red cells formed by the actively regenerating islands. Second, the erythrogenic elements may be injured to such an extent that they can produce only abnormally fragile cells which disintegrate rapidly. More plausible, perhaps, is the third possibility that the permanently damaged hematopoietic system is unable to produce mature erythrocytes in sufficient numbers to utilize the hemoglobin relinquished in the course of normal disintegration of red cells. Wallback, quoted by Hamilton,[1] attributed maturation-inhibiting qualities to benzene. Unused pigment would therefore accumulate in the reticulo-endothelial system. It seems probable that the destructive and also the inhibitory qualities of benzene were jointly contributory toward the development of the extensive hemosiderosis in the present case.

The marrow exhibited focal attempts at hematopoietic regeneration. This was particularly evident in the bodies of the vertebrae where there occurred coalescence of expanding cellular islands, a phenomenon which has been described by Selling.[3b] In certain foci the cellularity was sufficiently marked to simulate hyperplasia. That such changes may become diffuse has been shown in the Cabot case,[6] in Martland's case,

10. Martland, H. S.: Am. J. Cancer **15**:2435, 1931.

11. Piney, A.: Recent Advances in Hematology, Philadelphia, P. Blakiston's Son & Co., 1931, p. 219.

12. Long, P. H.: J. Clin. Investigation **2**:239, 1926.

cited by Hamilton,[1] and in Haldjman's case, recorded by Naegeli.[13] Selling [3b] noted totally hyperplastic marrows in his experimental rabbits within from ten to eighteen days after the administration of benzene was discontinued. The paradox of such hyperplastic marrows in the presence of blood pictures significant of aplasia is not uncommon. Only two of the thirteen cases in the series which Thompson, Richter and Edsall [14] reported in which there was the peripheral appearance of aregenerative anemia showed aplastic marrow. That the hyperplasia may progress to an extreme degree is demonstrated by the experimental production of polycythemia with benzene by Langlois and Desbrouis and by the observations of Delore and Borgman and Weil (cited by Hamilton [1]) on the development of leukemia in clinical benzene poisoning.

The changes in the spleen in this case were of extreme interest. Lymphoid tissue was apparently uninvolved except that it was displaced and compressed by the rapid growth of the intrasinusoidal cells. The extent and degree of the intrasinusoidal changes were, however, remarkable. Oxydase stains gave negative results, a fact which presupposed either extreme immaturity of the large cells described or relationship to the lymphoid or erythroid series. The absence of a significant degree of leukopoiesis and the histologic evidence of differentiation toward erythroblasts and normoblasts led to the identification of these cells as megaloblasts or less differentiated hematocytoblasts. Comparison of the cells with those in impression smears of bone marrow which had been stained with Giemsa stain led to the conclusion that the majority of the cells were megaloblasts. The direction of differentiation further confirmed this conclusion.

The metaplastic process was so marked that at first glance the possibility of neoplastic change was entertained. The absence of extrasinusoidal invasion, however, and the presence of definite though impeded maturation toward normoblasts precluded true neoplasia (fig. 2). Morris,[15] Reckzah (cited by Morris), von Domarus [16] and Selling [3b] all produced splenic hematopoiesis by using various toxic substances. In Andersen's [4] case marked splenic metaplasia was exhibited, which, however, was almost wholly granulocytic and differed further from this case in that the bone marrow was everywhere intensely hyperplastic. Donhauser [17] reported a case of bone marrow sclerosis with splenic changes

13. Naegeli, O.: Benzin und Benzolvergiftung, in Blutkrankheiten und Blutdiagnostik, ed. 5, Berlin, Julius Springer, 1931, p. 668.

14. Thompson, W. P.; Richter, M. N., and Edsall, K. S.: Am. J. M. Sc. **187**:77, 1934.

15. Morris, R. S.: Bull. Johns Hopkins Hosp. **18**:200, 1907.

16. von Domarus, A.: Arch. f. exper. Path. u. Pharmakol. **58**:319, 1908.

17. Donhauser, J. L.: J. Exper. Med. **10**:559, 1908.

simulating those found in the case reported here. In his case metaplasia occurred only in grossly visible nodules, and differentiation again was primarily granulocytic.

Scattered throughout the groups of splenic megaloblasts in this case were single large multinucleated megakaryocytes (fig. 4). These giant cells frequently exhibited multipolar mitoses and occasionally revealed phagocytic qualities. Such a picture is not altogether unique. Firket and Campos [18] described a case of benzene poisoning in which there was aplasia of the marrow and almost wholly megakaryocytic metaplasia in both the spleen and the liver. They were able to produce very similar changes in rabbits by means of saponin poisoning. Medlar [5] likewise showed increased production of megakaryocytes in rabbits poisoned with either benzene or saponin.

Considerable controversy exists concerning the mechanism of the initiation of extramedullary formation of blood. Gibson [19] suggested that it was purely compensatory. Pearce, Krumbhaar and Frazier [20] showed in their experiments on blood that splenectomized dogs exhibited slower recovery from induced anemia than normal controls and thereby implied that the spleen has compensatory blood-regenerative powers. Weiskotten, Schwartz and Steensland,[21] on the other hand, demonstrated that recovery from the anemia of benzene poisoning was neither hastened nor impeded by splenectomy. Obviously benzene introduced an additional factor. It has been shown by Selling [3b] that destruction of splenic parenchyma and that of marrow occur simultaneously in benzene intoxication and that subsequent regeneration is usually concomitant in both organs. It is not inconceivable that splenic destruction obviated compensatory activity in the benzene poisoning experiments. What then was basic in the production of the bizarre erythropoiesis noted in this case? Draper [22] and Brannan [23] each reported a case in which splenic and marrow hematopoiesis occurred simultaneously, and the former author inferred, probably correctly, that the splenic activity was supplementary and not compensatory. Foa, Tizzoni and Griffini, quoted by Howell,[24] observed that following partial splenectomy comparatively rapid regeneration of the spleen occurred. Accompanying this regeneration there was reappearance of the fetal property of blood formation. It is

18. Firket, J., and Campos, E. S.: Bull. Johns Hopkins Hosp. **33**:271, 1922.
19. Gibson, J. L.: J. Anat. & Physiol. **20**:324, 1885-1886.
20. Pearce, R. M.; Krumbhaar, E. B., and Frazier, C. H.: The Spleen and Anemia, Philadelphia, J. B. Lippincott Company, 1918.
21. Weiskotten, H. G.; Schwartz, S. C., and Steensland, H. S.: J. M. Research **33**:127, 1915.
22. Draper, G.: Bull. Ayer Clin. Lab. Pennsylvania Hosp., 1910, no. 6, p. 14.
23. Brannan, D.: Bull. Johns Hopkins Hosp. **41**:104, 1927.
24. Howell, W. H.: J. Morphol. **4**:57, 1890.

highly plausible that the destruction of the splenic parenchyma which occurs in benzene poisoning results in a similar resumption of hematopoietic activity. This undoubtedly varies in degree and kind with the extent of the damage inflicted.

The liver, as it so often does in cases of extramedullary hematopoiesis, in this instance also lagged behind the spleen in the amount of activity exhibited. Meyer and Heinecke [25] observed that in metaplasia the spleen shows earlier embryonic features than the liver. This was so both quantitatively and qualitatively in the present case.

Attention must be called in passing to the striking resemblance of the changes in the liver and spleen in this case to the lesion observed in erythroblastic anemia.[26] The appearance was quite similar, although in the latter disease a higher degree of cellular maturation is generally attained. Splenic and hepatic hematopoiesis occurring in the myelophthisic anemias, notably with extensive metastatic carcinoma in the bone marrow, is only superficially similar. In these anemias there is neither impairment of maturation nor limitation of blood development to a single type. It is this feature in conjunction with the neoplastic appearance and the evident deficiency of maturation noted which suggests that the extramedullary hematopoiesis in the present case is the result of anaplastic regeneration of damaged tissue rather than of simple compensatory metaplasia.

SUMMARY

The clinical history and the histologic observations in a case of benzene poisoning have been reviewed. The clinical picture was that of aplastic anemia. The disease was marked by the presence of leukopenia and severe anemia. The latter was benefited only transiently by repeated blood transfusions. The disease terminated fatally with gastro-intestinal hemorrhage. Associated with the anemia and the leukopenia were thrombocytopenia and macrocytosis. These were accounted for by the inadequate hematopoietic regeneration exhibited by the bone marrow. Low grade reticulocytosis and the presence of immature myeloid cells and nucleated red cells in the circulating blood were also explained on the basis of the changes occurring in the marrow. Massive metaplastic erythropoiesis was present in the spleen and to a less well marked degree in the liver. It is believed that the metaplastic changes observed were produced not as a compensatory mechanism but rather as the result of atypical regeneration in a liver and spleen intrinsically damaged by benzene.

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25. Meyer, E., and Heinecke, A.: Verhandl. d. deutsch. path. Gesellsch. **9**: 224, 1905.

26. Baty, J. M.; Blackfan, K. D., and Diamond, L. K.: Am. J. Dis. Child. **43**:667, 1932.

CHANGES IN THE LIVER IN AMEBIC DYSENTERY

WITH SPECIAL REFERENCE TO THE ORIGIN OF AMEBIC ABSCESS

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Since the excellent work of Councilman and Lafleur [1] in 1891 little has been written concerning the process of formation of early amebic abscesses of the liver. This is surprising because of the relatively high incidence of this complication. Craig [2] collected 1,529 cases of amebic dysentery that came to autopsy. Among these the frequency of abscesses of the liver ranged from 22 to 59 per cent with an average of slightly more than 40 per cent. The incidence of this condition is lower in nonfatal cases, for in another series of 1,429 cases of clinically diagnosed amebic dysentery collected by Councilman and Lafleur [1] only 21 per cent of the patients had hepatic abscesses. As is well known, approximately 50 per cent of amebic abscesses are found in the right lobe of the liver; in about 45 per cent of the cases they are multiple.

Amebas may reach the liver by one of three possible routes: 1. By direct extension through the bowel wall, the peritoneal cavity and the capsule of the liver. This is the theory of Councilman and Lafleur and is supported (*a*) by the finding of amebas in the peritoneal cavity,[3] (*b*) by the report of an amebic abscess of the abdominal wall in a patient suffering with amebic dysentery [3] and (*c*) by the frequent location of the abscesses near the capsular surface of the right lobe of the liver.[1] 2. By extension through the portal vein. Amebas have been frequently observed in the lumens of capillaries and venules near the intestinal lesions. 3. By extension through the lymphatics. This route is doubtful for obvious reasons, although Councilman and Lafleur report finding amebas in the lymphatics of the colon.[1]

Councilman and Lafleur [1] described the earliest amebic abscesses in the interlobular tissue about the portal vein and immediately beneath the capsule of the liver. These early lesions grossly resembled miliary

From the Department of Pathology, Northwestern University Medical School.

1. Councilman, W. T., and Lafleur, H. A.: Johns Hopkins Hosp. Rep. **11**: 395, 1891.

2. Craig, C. F.: Amebiasis and Amebic Dysentery, Springfield, Ill., Charles C. Thomas, Publisher, 1934, pp. 140-141.

3. Strong, R. P., in Osler, W.: Modern Medicine, Philadelphia, Lea & Febiger, 1925, vol. 2, p. 221.

tubercles more closely than small abscesses. Because there was no inflammatory reaction in the early stages the authors believed that these lesions were not true abscesses. They were among the first to observe the cytolytic action of amebas in the extension of lesions in the bowel and in the liver.

Craig [4] described early abscesses as areas of cytolysis in which red blood cells, a few leukocytes, connective tissue cells and granular débris are surrounded by an area of capillary hyperemia. The wall of the early abscess is infiltrated with leukocytes and frequently contains newly formed bile channels. The original bile ducts are often obliterated or encroached on by the wall of the abscess.

MacCallum [5] stated that the amebas lodge in the capillaries of the liver and "produce effects similar in principle to those set up in the intestine." He mentioned "small abscesses (from which the contents can be squeezed out like paint)" which "appear as opaque, yellowish-white areas occupying the space of one or two lobules."

In view of the scanty literature on the origin and mode of formation of amebic abscesses of the liver, the following case is presented.

A white man 55 years of age was admitted to the Alexian Brothers Hospital Sept. 5, 1936, with a diagnosis of pulmonary tuberculosis.

He had enjoyed good health until the latter part of the preceding July. At that time he became warm and drank some cold beer. A few hours later he had a severe chill and had not been well since. He entered complaining of a sharp pain over the region of the gallbladder and in the right side of the chest, a productive cough with stringy mucoid sputum, nausea and vomiting once or twice a day, shortness of breath and a loss of 30 pounds (13.6 Kg.) in weight. He was also constipated.

In childhood he had typhoid fever and measles; twenty years ago he had gonorrhea and at the time of examination he had an untreated hydrocele and varicocele.

The blood pressure was 125 systolic and 85 diastolic. The pupils were sluggish to light; the pharynx was injected. There was dulness over the right middle and lower pulmonary lobes, with roughened breath sounds. The right upper quadrant of the abdomen was tender without rigidity or muscle spasm.

Urinalysis gave negative results; the sputum was mucoid and contained many pus cells and chains of streptococci but no tubercle bacilli. A few microscopic red blood cells and much mucus were found in the feces, but no amebas. The hemoglobin content was 80 per cent; the red blood cells numbered 3,360,000; the white cells, 15,200; the differential count showed 68 per cent polymorphonuclears, 22 per cent small lymphocytes and 5 per cent large lymphocytes. The Wassermann and Kahn reactions were negative. An x-ray picture taken September 28 revealed a pleuritic effusion on the right side, with a dense shadow, 3 fingers in width, extending upward from the diaphragm. The lungs themselves appeared normal.

Diagnosis.—Tuberculosis of the right lung, with the necessity to rule out (1) subphrenic abscess, (2) cholecystitis and (3) malignant tumor.

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4. Craig,[2] pp. 108 and 110.

5. MacCallum, W. G.: A Textbook of Pathology , ed. 6, Philadelphia, W. B. Saunders Company, 1936, p. 811.

October 3, a partial resection of the ninth rib posteriorly was done and a pneumothorax was created. Pus was observed exuding through the diaphragm. A lower incision was then made for drainage, and rubber drains were inserted.

Following this operation the patient improved temporarily and then began to follow a slow downhill course. He died November 6, three and a half months after the onset of the symptoms.

Autopsy.—The body was that of a poorly nourished white man, dead six hours. The only significant changes were found in the colon, liver and right thoracic cavity. Other organs were essentially normal. In the right posterior axillary line were two unhealed surgical incisions, 5 and 6 cm., respectively, about opposite the eighth and ninth ribs. The right lung was collapsed. The diaphragm was pressed upward to the level of the third rib by the enlarged right lobe of the liver and was firmly adherent to the wall of the chest below the incisions just mentioned. The lining of the right pleural cavity was everywhere red, and a good part of it was covered with a thick yellowish-white exudate.

The abdominal cavity contained about 25 cc. of turbid fluid chiefly in the pelvis. The peritoneum was smooth and glistening but irregularly red. The entire colon was thick and reddened, and in the mucosa there were numerous typical amebic ulcers of irregular shape, with undermined edges, and varying from 3 mm. to 6 cm. in diameter. Microscopic examination of the material scraped from these ulcers revealed many sluggishly active amebas, and in properly stained sections numerous amebas were found.

The right lobe of the liver was enormously enlarged upward, and the diaphragm was firmly adherent to the posterior part of its upper surface. A rubber drain extended from the thoracic wound through the diaphragm and into a large cavity in the right lobe of the liver. When the liver was sectioned, three large abscess cavities were found (fig. 1 *B*). One of these had been drained through the incisions in the wall of the chest. Two centimeters medial to the drained abscess there were two separate abscesses, each about 10 cm. in diameter and filled with a thick stringy pale yellowish brown pus. The walls of these abscesses were yellow-gray and up to 5 mm. in thickness, and the inner linings were yellowish brown and shaggy.

On the anterior surface of the right lobe there were two areas slightly darker reddish brown than the surrounding tissue and measuring 2 by 5 and 3 by 5 cm. Surfaces made by sectioning through these areas revealed dark red-brown to purple-brown, sharply demarcated wedge-shaped areas extending 5 and 7 cm., respectively, into the hepatic parenchyma (fig. 1 *A*). Within these darker areas there were numerous pale yellow-white patches from pinpoint size to 2 by 5 mm. The larger of these had a leaflike configuration as if they followed the branching of the portal veins. The remainder of the hepatic parenchyma was pale purple-brown with lighter yellow-brown lobular markings.

Microscopic Examination.—The central veins of the hepatic lobules and the adjacent sinusoids were moderately dilated, and Kupffer cells in this region frequently contained yellow-brown pigment granules. The hepatic cells in the midzonal portion of the lobules contained many fat vacuoles; those nearest the triads were moderately swollen, and their cytoplasm was granular. Throughout the liver the portal connective tissue was irregularly increased, some triads showing marked thickening with lymphocytic infiltration, while others were unchanged. Adjacent to the larger abscesses, the portal connective tissue was markedly increased; the liver cell cords were compressed and atrophic and ran parallel to the wall of the abscess; the sinusoids were dilated. This was especially marked between the wall of the abscess and the thickened capsule of the liver.

The wall of the larger abscess was composed of dense fibrous connective tissue, which at the periphery often encircled small islands of liver cells. Bile ducts were numerous throughout the wall of the abscess, especially in the outer margins. Toward the inner surface of the wall there were increasing numbers of lymphocytes and monocytes between the fibers of the connective tissue. The contents of the cavity of the abscess consisted of much cellular débris, polymorphonuclears, monocytes and lymphocytes, enmeshed in an irregular fibrinous network. In the wall of the abscess an occasional ameba was found. The wall of the drained abscess ranged up to 2 cm. in thickness, and it was similar in structure to the walls of the abscesses

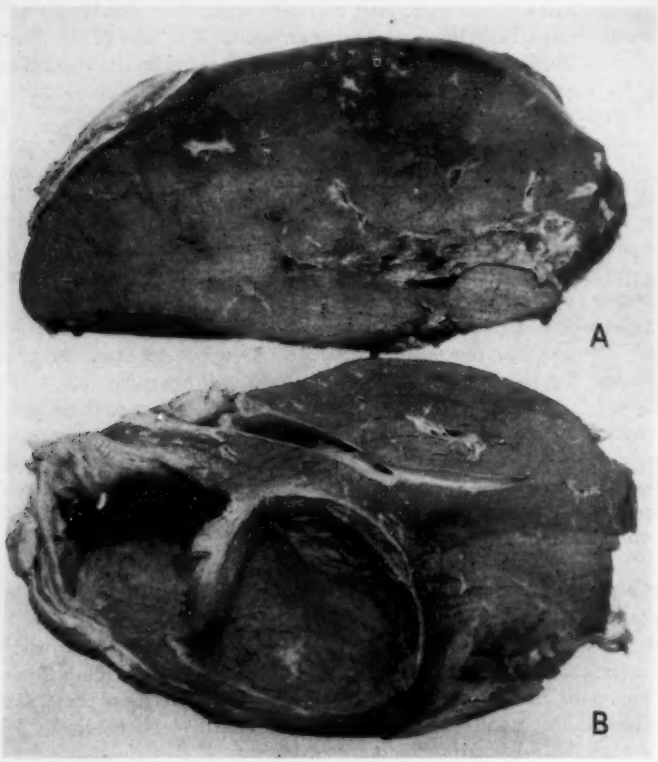

Fig. 1.—Liver from the case reported in detail: *A*, the anterior part of the right lobe with two infarct-like areas which contain multiple small amebic abscesses. *B*, the posterior part of the right lobe with three large abscesses one of which is collapsed.

previously described with the exception that infiltrating the wall there was a greater number of pus cells. The contents of this abscess consisted of cellular débris rich in polymorphonuclear leukocytes.

Sections from the wedge-shaped deeper brown areas in the liver have grossly a moth-eaten appearance owing to the presence of abscesses from 1 to 3 mm. in diameter, which extended irregularly from the finer portal triads into the surrounding liver tissue (fig. 2 *A* and *B*). Some of these coalesced to form larger branch-

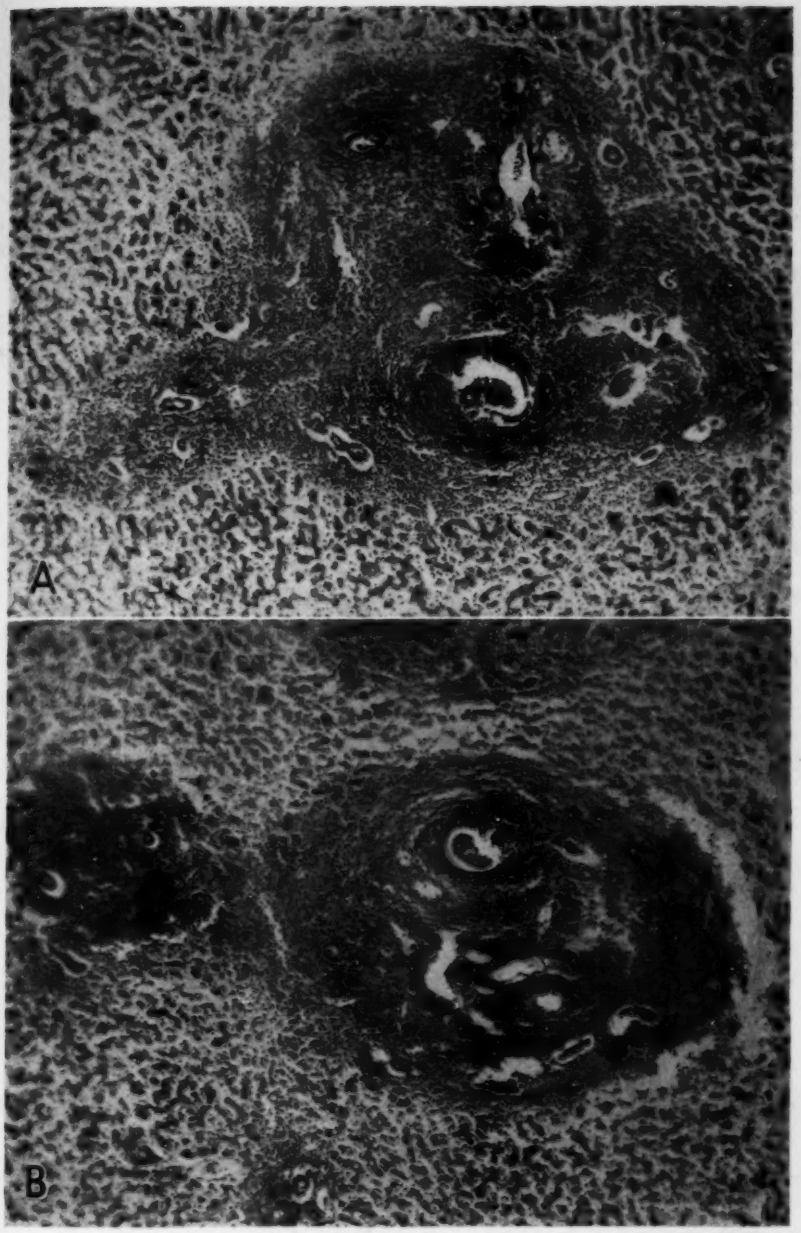

Fig. 2.—*A*, very early amebic abscess beginning in small triad, showing lobulated branching form. The hepatic artery is distinct. A bile duct near the right border is partially eroded. The portal vein in the left center is barely recognizable. Amebas are present throughout the abscess but are most numerous in the pale area in the left upper quadrant. There is apparent new bile duct formation at the periphery. Shaudinn fixation; iron-hematoxylin stain; × 75 (reduced).

B, very early amebic abscess beginning in a small triad with three smaller abscesses in nearby triads. The hepatic artery and bile duct are distinct. The ragged area in the right half of the abscess is the former site of the portal vein. Fixation, staining and magnification as in *A*.

ing abscesses. The central parts of these abscesses were composed of cellular débris with lymphocytes and monocytes and remains of blood vessels and bile ducts. Amebas were found in all of the small abscesses studied. They were present in the left segment of figure 2 *A* and in the right half of figure 2 *B*. The large number of lymphocytes present was probably due to infiltration of the triad with these cells (as described in a preceding paragraph) before the arrival of the amebas. In some places the hepatic cells bordering these small abscesses stained poorly, were finely granular and contained vacuoles, and the nuclei of many of them were either very pale or pyknotic. This was well shown along the left border of the abscess in figure 2 *A*. In the abscesses shown in figures 2 *A* and *B* the arteries and bile ducts, even with partially eroded epithelium, were easily identified, but the portal vein was destroyed almost beyond recognition. All of the small abscesses were well below the capsule of the liver near the center of the wedge-shaped area.

LIVER CHANGES IN NINETEEN CASES OF AMEBIC DYSENTERY

Little attention has been given to the changes in the liver outside the immediate sphere of influence of an amebic abscess or to the changes found in the liver in the presence of active amebic intestinal lesions but with no definite abscess formation. For this reason changes in the liver in 18 additional cases of amebic dysentery have been studied. The significant facts in the 19 cases are presented in the table.

The cases came to autopsy at the Cook County Hospital, Alexian Brothers' Hospital and Evanston Hospital during seven years from 1929 to 1936. Drs. R. H. Jaffé, J. P. Simonds and F. D. Gunn gave permission for the presentation of summaries of these cases.

The ages of the patients ranged from 31 to 72 years; there were 16 white men, 2 colored men and 1 white woman. Complicating the amebic dysentery in 7 cases was peritonitis; in 2, syphilis; in 1, carcinoma with metastases to the liver; in 1, lymphosarcoma, and in 1, vegetative endocarditis. Thirteen of the 19 patients had abscesses of the liver; in 12 they were in the right lobe, and in 5 the abscesses were multiple. In 3 of the cases the capsule of the abscess was thin (less than 3 mm.); in the remainder the walls varied from 4 mm. to 2 cm. in thickness. In only 6 of the cases was a diagnosis of amebic dysentery made clinically. These patients gave histories of symptoms covering periods of from ten days to three years, the average being a little over two months. In 12 of the 19 cases, the liver was larger than normal; in 4 it was of normal weight; in 2 it weighed less than normal, and in 1 the weight or size of the liver was not recorded.

In 18 of the cases the portal connective tissue was increased, varying from marked diffuse to moderate irregular or spotty increase. In the 9 cases in which the fibrosis was more marked, definite proliferation of the bile ducts was present. There was a noticeable increase in lymphocytes and monocytes in the portal triads in 10 cases. Fatty changes, present in 9 cases, were most marked in the region midway

Data on Nineteen Cases

Case	Patient's Age	Race	Sex	Size of Liver	Complicating Pathologic Conditions	Duration of Symptoms	Hepatic Abscess	Multiple Abscesses of Liver	Right Lobe	Other Lobes	Deep	Superficial	Thick-Walled Abscesses	Thin-Walled Abscesses	Increase in Portal Connective Tissue	New Bile Duct Formation	Fatty Change	Pigment in Hepatic Cells	Lymphocytic Infiltration
1	34	Colored	M	Large	Pneumonia	?	+	0	+	..	+	..	+	0	..	+	..	..	..
2	72	White	M	Small	Perforation of abscess and bowel with diffuse peritonitis	2 wk.	+	+	+	+	+	+	0	+	0	..	..	+	+
3	56	White	F	.....	Pulmonary embolus	1 yr.	+	0	+	..	..	+	+	0	+	+	..	..	..
4	45	White	M	Large	Rupture of hepatic abscess	3 wk.	+	+	+	+	+	+	+	..	+	+	..	..	+
5	64	White	M	Large	.....	8 wk.	+	0	+	..	+	0	+	0	+	+	..	..	..
6	35	White	M	Large	Pneumonia; peritonitis	4 mo.	+	0	+	0	+	0	+	0	+	+	+	+	..
7	40	White	M	Large	Operative drainage of hepatic abscess	5 mo.	+	0	+	..	..	+	+	..	+	+	..	+	..
8	49	White	M	Large	Operative drainage of hepatic abscess	3 yr.	+	+	+	0	+	..	+	..	+	+	+	..	+
9	31	White	M	Large	Vegetative endocarditis	1 mo.	+	0	0	+	+	0	+	0	+	..	+	+	+
10	58	White	M	Large	Lymphatic leukemia; alcoholic cirrhosis of liver	1 mo.	+	+	+	0	+	0	+	0	+	..	..	..	..
11	61	White	M	Large	Carcinoma of colon with metastasis to liver	3 mo.	+	0	+	0	+	0	+	0	+	..	..	..	+
12	45	White	M	Normal	Pneumonia	4 mo.	+	0	+	0	+	0	+	0	+	..	..	..	..
13	55	White	M	Large	Right empyema, surgical drainage	3 mo.	+	+	+	+	+	+	+	+	+	+	+	+	+
14	53	White	M	Large	Pneumonia	1 mo.	0	..	..	..	..	..	..	..	+	+	+	+	+
15	55	White	M	Small	Tuberculous bronchopneumonia; multiple scars of liver; hepar lobatum	1 yr.	0	..	..	..	..	..	..	..	+	+	+	+	+
16	59	White	M	Large	Peritonitis	2 wk.	0	..	..	..	..	..	..	..	+	+	+	+	+
17	55	White	M	Normal	Perforation of bowel; peritonitis	9 mo.	0	..	..	..	..	..	..	..	+	0	+	+	+
18	67	Colored	M	Normal	Arteriosclerotic gangrene of leg; syphilis	1 mo.	0	..	..	..	..	..	..	..	+	..	..	..	..
19	67	White	M	Normal	.....	3 wk.	0	..	...	..	..	..	..	..	+	..	+	+	..
Total	.....					.....	13	5	12	4	11	5	12	2	18	11	9	10	10
Percentage	.....					.....	68	38	60 92	30	84	38	92	15	94	58	47	53	53

between the central vein and the portal triads. Pigment in the liver cells or the Kupffer cells or both was prominent in 10 cases. The increase in fibrous tissue was most marked in the region adjacent to the large abscesses, and in the periphery of their walls isolated islands of liver tissue were frequently found. Generally the hepatic parenchyma was compressed and atrophic near the large abscesses, and the hepatic cell cords had a tendency to lie parallel to the capsule of the abscess.

In only one of the cases with multiple amebic abscesses were the walls thin, and there was little or no increase in the periportal connective tissue. In this case the symptoms were of only ten days' duration, and the patient was 72 years of age with complications of bilateral bronchopneumonia and diffuse peritonitis. In this case the disease was probably of too short duration to allow the typical proliferative changes to take place. An interesting feature of this case was the presence of amebas in a thrombosed branch of the portal vein in the liver[6] (case 15 of table).

COMMENT

The wedge-shaped areas in the liver containing the small amebic abscesses in the case reported here in detail had the gross appearance —shape and color—of infarcts, although, as usual in such lesions in the liver, the hepatic cells were viable. Since the amebas reach the liver by way of the portal vein, it is to be expected that the branch in which they find lodgment will become thrombosed. In one of the cases studied (no. 15 in table) an ameba was actually found in such a thrombus.[6] The shape and distribution of the lesions in figures 1 *A* and 2 *B* give the impression that the amebic infection of the liver in this case began in a branch of the portal vein, extended along its subdivisions toward the capsule and spread through the connective tissue of the triads into the parenchyma of the liver. It is probable, therefore, that in this way "infarction" due to intrahepatic portal thrombosis is an important factor in the production of large amebic abscesses of the liver.

In 18 of the 19 cases studied the periportal connective tissue was distinctly increased. This increase was either diffuse or patchy in distribution. Especially the patchy fibrosis may represent foci of lodgment of amebas, with the production of only small abscesses which healed. It is possible, in other words, that in the course of amebic dysentery many amebas reach the liver and cause only slight local damage and scarring without the formation of large abscesses.

6. Jaffé, R. H.: Personal communication to the author.

SUMMARY

Amebas gain entrance to the liver by way of the portal vein, and abscesses originate in the portal triads. Amebas have been observed by others invading the capillaries near the intestinal lesions, and they were seen in a thrombosed portal vein in the liver in a case reported here. The early abscesses were found well below the capsule of the liver in 84 per cent of the cases in this series.

The very early abscesses begin as areas of lysis extending into the liver parenchyma from the portal triads, with little accompanying inflammatory reaction, and frequently increase in size by coalescing with neighboring abscesses. The central area of liquefaction is bordered by a thin meshwork of fibrinous strands in which are a moderate number of lymphocytes and large mononuclear cells. Polymorphonuclear leukocytes are rarely found. Immediately about the small abscesses the hepatic sinusoids are usually moderately engorged with red blood cells.

Increase of portal connective tissue plays an important part in walling off the larger abscesses. It is often present in diffuse or focal distribution even in cases with active intestinal lesions but without abscess formation. The focal type of fibrosis may be scar tissue residues of healed small amebic abscesses. In the periphery of the walls of the larger abscess, islands of hepatic cells were frequently found surrounded by fibrous tissue. Increase in the number of bile ducts in this region was also common.

Definite hepatitis is associated with active amebic lesions in the colon. This is indicated by the generalized increase in the portal connective tissue in all but one of the cases reported here, by lymphocytic and mononuclear infiltration and by the presence of constant degenerative changes of variable type—parenchymatous degeneration, fatty changes, hemosiderosis and lysis of hepatic cells.

FAT IN THE INFANT BRAIN IN RELATION TO MYELIN, BLOOD VESSELS AND GLIA

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The significance of fat in the infant brain has been the subject of much controversy among German investigators since Virchow [1] in 1867 first published his observations on fat-laden glia cells in the white substance. He believed them to be manifestations of pathologic changes in a disease which he called congenital encephalitis. Hayem [2] questioned the pathologic derivation of the fat since he found it in all of twelve infants, but thought the number was perhaps too small for any conclusions. Other investigators agreed with Virchow, but in 1870 Jastrowitz [3] studied many brains of fetuses. new-born infants and infants several weeks of age. The presence of fat in all these led him to the conclusion that the fat must be of physiologic importance in the formation of myelin, since the areas of predilection were those of the white substance of the hemispheres and the cord. He thought the cells were young glia cells which, passing through a fat granule stage, took up fat as a by-product of myelin, and later disappeared. Virchow [4] opposed this view in published work and open discussion on the ground that fat was not present in the brains of all infants, that it was confined to certain places when present, and that the transformation of glia cells into fat granule cells and back again into normal glia cells seemed impossible. Later Merzbacher [5] agreed with Jastrowitz, and in twenty-six new-born infants distinguished nine different types of physiologic fat granule cells. Ceelen,[6] following Fischl's [7] idea that small round cells about the ventricles were inflammatory and thus linked to Virchow's encephalitis, also endeavored to prove that the fat was pathologic because of these cells. Later he retracted his statement as to

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From the Richmond Memorial Hospital.

1. Virchow, R.: Virchows Arch. f. path. Anat. **38**:129, 1867.
2. Hayem, G.: Sur les diverses forms d'encéphalite, Paris, A. Delahaye, 1868.
3. Jastrowitz, A. L.: Arch. f. Psychiat. **3**:162, 1871.
4. Virchow, R.: Berl. klin. Wchnschr. **4**:705, 1883.
5. Merzbacher, L., in Nissl, F., and Alzheimer, A.: Histologie und histopathologische Arbeiten über die Grosshirnrinde mit besonderer Berücksichtigung der pathologische Anatomie der Geisteskrankheiten, Jena, Gustav Fischer, 1909, vol. 3, p. 1.
6. Ceelen, W.: Virchows Arch. f. path. Anat. **27**:152, 1920.
7. Fischl, R.: J. f. Kinderh. **49**:58, 1899.

the pathologic significance of both the fat granule cells and the round cells, considering the latter embryonic foci. Wohlwill,[8] from the examination of seventy-seven brains, concluded that the fat was physiologic, taking part in the building up of myelin. He also thought that the size of the fat droplets and the morphology of the cells were distinguishing features of pathologic and physiologic fat. Guillery [9] next stated that the fat-laden cells of infants are all alike and although morphologically similar to those observed in brain injury in adults, they are physiologic in significance, since they are present in all brains to the eighth month of life, when myelinization is complete. Schwarz [10] concluded that since the fat cells of infancy were similar to those observed in traumatic lesions of the brain in adults, they were pathologic. Rydberg [11] was able to show, using a combination of a silver stain and a stain for fat, that fat granule cells were present only in injured brains and that in uninjured brains the fat accumulates around the astrocytes. Roback and Scherer [12] doubted the physiologic interpretation of the fat but disagreed with Schwarz' theory of brain lesions.

MATERIAL AND METHODS

In the present study of fat and myelin formation, the brains of forty-six infants were examined. Twenty-five of the infants were less than 6 months old; the others were of various ages up to 2 years. As the myelin sheaths are generally well developed in the cord, medulla oblongata, pons, cerebral peduncles, internal capsule and basal ganglions at birth, frozen sections were not made of these. Blocks were cut from the occipital lobe, including the area striata, and from the cornu ammonis, the frontal and central parts of the cortex and the midportion of the cerebellum. The stains used were those of Cajal for astrocytes, Spielmeyer's stain for myelin sheaths and the silver carbonate stain of Hortega combined with Herxheimer's stain for fat. Pyroxylin (celloidin) sections were also made of the basal ganglions and stained with toluidine blue.

OBSERVATIONS

Myelin and Fat.—At birth, the external optic fibers, the B fibers of the cornu ammonis and the fibers of the lower part of the centrum ovale in the central area are fairly well myelinated, as are some of the fibers of the corpus callosum, area striata, gyri and upper part of the centrum ovale of the central areas. Exceptions were found in two infants aged 17 and 19 days, in whom myelin had not yet developed in the upper part of the centrum ovale and gyri of the central areas; in another infant, aged 2 days, the B fibers were but partially myelinated, and in another, aged 10 days, the fibers in the upper part of the centrum ovale and gyri of the area striata had but few sheaths. Fat at birth and in the first month is heavily distributed in the corpus callosum, in the white substance of the lower

8. Wohlwill, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. **68**:383, 1921.
9. Guillery, H.: Ztschr. f. d. ges. Neurol. u. Psychiat. **84**:205, 1923.
10. Schwarz, I.: Ztschr. f. d. ges. Neurol. u. Psychiat. **90**:267, 1924.
11. Rydberg, E.: Acta path. et microbiol. Scandinav., supp. 10, 1932, p. 1.
12. Roback, H. N., and Scherer, H. J.: Virchows Arch. f. path. Anat. **294**: 365, 1934.

part of the gyri and in most of the centrum ovale. It is less marked along the external optic fibers, the well myelinated B fibers and the fibers of the lower part of the centrum ovale of the central areas. Little fat is demonstrable at any time in the upper part of the white substance of the gyri and none directly below the cortex. As can be seen in figure 1, showing the upper part of the centrum ovale in the first month, it occurs as small globules between and along the processes and around the cytoplasm of the microglia cells, oligodendroglia cells and astrocytes. Occasionally it is formed around the ganglion cells. It also lies free in the tissue and may overlay the cytoplasm and nuclei of the cells, the

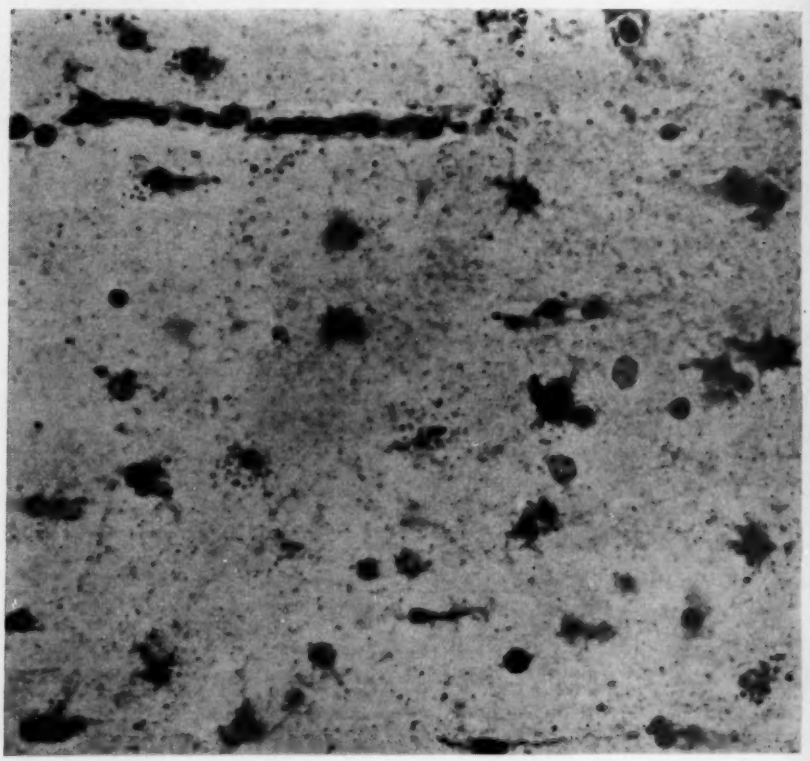

Fig. 1.—Upper part of the centrum ovale in the first month of life, prepared with a stain for fat plus a silver stain. Numerous fat globules are stained as dark particles in the tissue and around the oligodendroglia cells, microglia cells and particularly astrocytes and their pseudopodia, the latter often without visible nuclei and vice versa. Myelin has not developed.

chief of which are the astrocytes. The globules of fat along the pseudopodia are also shown in the photograph, the smaller ones at the vascular attachment. Even the pseudopodia of the spongioblasts are partially surrounded by globules. Not very much collects about the microglia or oligodendroglia cells; most of these cells show no fat. Fat is pronounced around the larger vessels in the lower part of the centrum ovale and corpus callosum but is slight in the other parts of the

white substance. Figure 1 shows how few globules are present in the spaces about the smaller vessels of the upper part of the centrum ovale at this time.

At two months, the progress of myelinization is likewise variable. In two infants it had developed little since birth. In the others, the myelinization of the fibers of the corpus callosum, external optic tract, area striata and central areas was well advanced. At this age the fat has decreased in these places except around the glia cells and vessels of the upper part of the centrum ovale and in the adventitial spaces of the larger subcortical vessels, the fat in the latter having

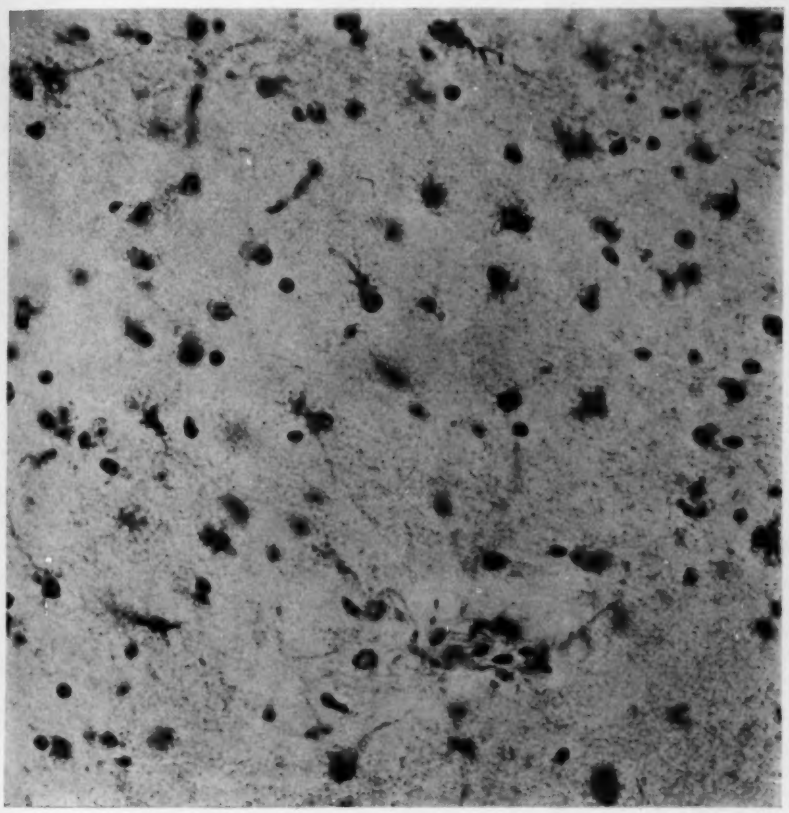

Fig. 2.—Lower part of the centrum ovale at seven weeks of life, prepared with a stain for fat plus a silver stain. There is fair myelin formation. Less fat is present than in the upper part of the centrum ovale as shown in figures 1 and 3. Young astrocytes with large clear round polar nuclei and subnuclear cytoplasm with fine processes and pseudopodia are shown; also, cells with apparently dark lentil-shaped nuclei and processes and the small round dark nuclei of medulloblasts and oligodendroglia cells.

increased. It is evident that the fat in the upper part of the centrum ovale at one month shown in figure 1 is much greater than that in the lower part of the centrum ovale of the central area at seven weeks shown in figure 2. At this age

there are fibers with myelin sheaths also in the upper part of the centrum ovale and the white substance of the other gyri, the internal optic tract, other subventricular tracts and the lower part of the centrum ovale of the frontal area. The fat is as heavy as in the first month, while it has increased in the larger subcortical vessels.

At three months, few fibers are found without myelin sheaths. Yet in one infant with intestinal toxemia myelinization had not advanced beyond that of the first month, and the accumulation of fat was marked. In well myelinized areas at this age, only a few glia cells and vessels are found with fat, and these in the upper part of the centrum ovale, although the adventitial fat in the subcortical vessels is still pronounced. Fat is abundant, however, although of variable quantity, in

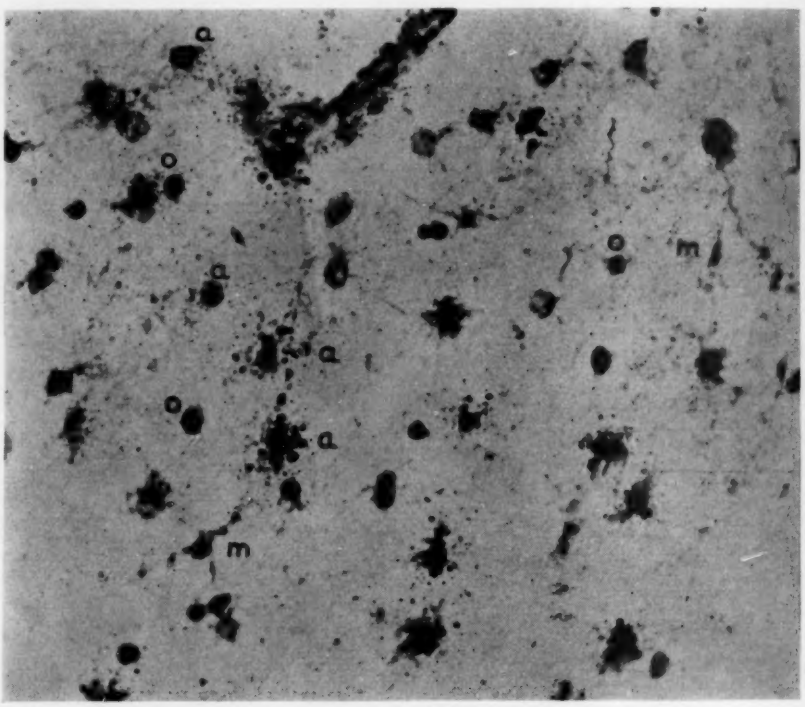

Fig. 3.—Upper part of the centrum ovale of the frontal area in the fourth month of life, when myelin formation is incomplete. The glia cells with fat are less numerous than in figure 1. The oligodendroglia cells have enlarged and increased in number as compared with those found at the first month. The letter *a* indicates astrocytes, *o* oligodendroglia cells and *m* microglia cells. A stain for fat was combined with a silver stain.

the upper part of the centrum ovale, in the white substance of the lower part of the gyri as well as in the adventitial spaces of the subcortical vessels, wherever the myelin sheaths are but partially developed. Figure 3 is a photograph of the upper part of the centrum ovale in the frontal area, in which the myelin is fairly well developed at three and a half months, while the fat is distinctly less than in the upper part of the centrum ovale during the first month (fig. 1), when myelin

is not demonstrable, and more than in the lower part of the centrum ovale at seven weeks (fig. 2), where the myelin sheaths are better developed.

At the fourth month, the myelin is apparently completely formed; i. e., at this and subsequent periods no unstained areas or fibers are found. The globules of fat, which were demonstrable in all of twenty-one infants to the fourth month, are present at this time only as perivascular fat in the larger subcortical vessels. The latter could be observed in all infants to the age of 2 years.

The first scattered fibers in the cortex to show myelin are found at three and a half months, while full myelinization apparently takes place at the ninth month. In this series fat was never demonstrable in the cortex at any time, nor was it ever present in the few periventricular nuclei that were stained, nor in the subventricular glial recesses. In the cerebellum, the fibers of the central white substance and a few fibers of the lamellae are myelinized at birth. Few fat globules can be found in the central part either before birth or at birth and none later. Nor are there ever fat globules in the lamellae. The latter have full development of the myelin sheaths in the white substance between the third and fifth months. Fibers in the granular layer were stained at the age of 6 months although absent in an infant of 7 months.

Glia Cells.—The astrocytes of the white substance of the hemispheres are numerous at birth, particularly in the lower part of the centrum ovale, but the number per field diminishes as the brain enlarges. Both mature and immature cells are scattered indiscriminately, although mature star figures are few, as figure 1 shows. The least developed ones are also few and appear as small round or oval nuclei, each with a thin scarcely perceptible cytoplasmic tail attached to a vessel. The majority are fairly mature, with varying amounts of subnuclear cytoplasm, from which spring fine enveloping processes, also pseudopodia that are short and thick or long and slender and single or double. The nuclei stain lightly or heavily. They are generally large and round, although some may be of lentil shape. The latter are prominent if the time post mortem is long. Not many of the immature astrocytes are distinctly shown in figures 1 and 2, since it is difficult to bring the entire cell into focus or to find them all lying in the same plane when they are numerous about the vessels and attached from all directions, with curved and twisted pseudopodia. As can be seen in the photographs, the nuclei appear to be without cytoplasm or pseudopodia, while the latter are also solitary. With age they tend to lie more evenly. They are mature by the eighth month, but an occasional young cell may be observed to the twelfth month.

The oligodendroglia cells of the centrum ovale appear chiefly as young cells, each with a small dark nucleus, round or somewhat oval, and scanty cytoplasm, which stains poorly. During the first two months, they are easily confused with the small dark round nuclei of the indifferent cells, or medulloblasts, in the lower part of the centrum ovale. By the fourth month, the nuclei of the oligodendroglia cells are uniform in size and less hyperchromatic, while the cytoplasm of each stains as a sharply demarcated large clear ring. Their scarcity in the white substance at birth and during early infancy is in sharp contrast to the large number in the adult brain. A relative estimation of their number in relation to the astrocytes is difficult to obtain in the lower part of the centrum ovale because of the medulloblasts and the bunching of the astrocytes around the vessels. The irregularity of the cell groups and the polymorphism of the nuclei are evident in figure 2 of the lower part of the centrum ovale. Although both the oligodendroglia cells and the astrocytes are near the vessels in the upper part of the centrum ovale and in the white substance of the gyri, they appear evenly distributed, as figure 1 shows. The ratio of oligodendroglia cells to astrocytes

averages 1:2 during the first two months, a ratio which is fairly well represented in figure 1. The number varies somewhat in each brain to the fourth month, when the astrocytes are about one and a half times as many as the oligodendroglia cells. The slight increase in number of the oligodendroglia cells can be seen in figure 3. At six months of life they equal the astrocytes in number; then the oligodendroglia cells begin to outstrip the astrocytes, although inconstantly to the tenth month, when the ratio is 1.5:1. In the second year their number continues to increase with some irregularity until they are more than twice as numerous as the astrocytes, yet are fewer than in the adult brain.

The microglia cells, also, are not numerous at birth, and their number does not increase much during the first two years. In the early months are found multipolar cells and immature bipolar cells, as figure 1 shows.

Many of the astrocytes in the white substance of the cerebellum are mature at birth, but spongioblasts are present in the first month and young astrocytes to the fourth month, when all are apparently mature. They are numerous in each field in the first month, but the numbers diminish gradually with age. The oligodendroglia cells are few and chiefly small in the first month. They reach a mature size by the third month. They equal the astrocytes in number at the fifth month and then become more numerous, although not attaining the adult number at the end of two years. The microglia cells are infrequent during the first two years and are chiefly multipolar in the first month, with but few bipolar ones.

The astrocytes of the cortex are spread over the layers without any recognizable difference in their distribution and show about the same number per field as is found in the adult. Those in the upper layers are chiefly mature radiating forms at birth, but many in the lower layers are immature, with polar nuclei. The latter may be present at seven months. The oligodendroglia cells are few and small during the first two months. The microglia cells are also less numerous than the astrocytes, and some are bipolar. Together with the oligodendroglia cells, they increase as trabant cells but are less numerous at the end of two years than in older brains. The oligodendroglia cells are more prevalent in the lower layers; the microglia cells appear equally in all layers.

The predominating cells in the basal ganglions and internal capsule during the first three months are the astrocytes. Many are mature. They remain fairly constant in number per field as the brain enlarges. The few microglia and oligodendroglia cells increase in size and number, but the latter do not attain the adult proportion in two years.

COMMENT

As fat was found in all the brains to four months, when it ceased abruptly, it is not possible to assign a pathologic significance to it. Rydberg [11] and Sjövall [13] attempted to explain the fat by suggesting that the astrocytes, around which it accumulates, are the carriers in an interneural circulation. They believe that fat is generally taken from the vessels and assimilated unseen, but when the vitality of the astrocytes is diminished, or the interneural circulation is delayed by too much material, as in the large supply of fat for myelinization, it is precipitated as neutral fat and left lying around both the vessels from which it had been taken and the astrocytes which had absorbed it. Their theory ignores the objection first advanced by Virchow against a physiologic

13. Sjövall, E. S.: Acta path. et microbiol. Scandinav., supp. 11, 1932, p. 79.

interpretation of the fat and reiterated more than fifty years later by Schwarz as his chief counter-argument, namely, the absence of fat during the myelinization of the medulla oblongata, pons, internal capsule, cortex and cerebellum, areas which also tax the astrocytes to supply fat. Certainly as much fat must be needed in the internal capsule and cerebellum as in the external optic fibers and those of the gyri, yet the former do not suffer a poor interneural circulation with a precipitation of fat. Schwarz' opposing belief in a brain injury cannot be upheld either, since the fat granule cells, which are the substitutes for destroyed blood vessels in brain injury, are not present. Moreover, he does not answer his own objections, as injuries sustained at birth are not confined to the centrum ovale or to the corpus callosum.

Roback and Scherer, also, reject the theory of a relation between the fat and myelin, not only on the grounds stated by Schwarz, but because they could find no progressive diminution of fat with age. In the present study, immature states, in which the fat was pronounced and the myelin undeveloped, were found at birth and at two and three months. This variation in growth seems to account for their observation that similar amounts of fat may occur in a stillborn and an older infant. Moreover, it is only when myelinization is advanced that fewer cells with fat are found in either the upper or the lower part of the centrum ovale. Wohlwill thought that since the time of the persistence of fat was unknown one could not say that it decreased with myelinization. Yet different tracts in the same microscopic field and in different parts of the same brain may be compared with relation to the degree of myelinization and accumulation of fat. It is thus possible to observe during the first month a lesser amount of fat in the myelinized external optic tract than in the internal optic tract, which is not myelinized. In the second month the contrast is even more striking, since the decrease is marked in the external optic fibers while the fat is still heavy in the internal optic tract, in which myelin is just beginning to develop. The earlier loss of fat in the area striata and the central areas, which form myelin before the adjoining gyri do, can also be demonstrated. However, as against the belief that the fat is physiologic, Roback and Scherer have suggested that the toxicity of the diseases in early infancy decomposes the myelin into neutral fat. This proposal does not explain either the areal limitations of the fat deposits or their constant formation regardless of the toxic agent.

The perivascular fat is considered by Rydberg and Sjövall as part of the stagnant neutral fat used in the formation of myelin. But this neutral fat persists in the adventitial spaces of the subcortical vessels from the first month through the first two years and later. Wohlwill regarded it as a product of metabolic disturbance due to fever, yet it can be found in cases without fever. Its continuance in the

adventitial spaces of the larger subcortical vessels after myelinization has ceased suggests that it is a waste product in metabolism involving the myelin at the junction of the gray and white substances. Some of these vessels are present in the upper part of the gyri, where little or no fat is found about the glia cells during the time of myelinization. This seems to indicate that during this time as well as in the later months fat may slowly find its way to the adventitial spaces of the larger vessels, where its removal is also a slow procedure. Since there is nothing to distinguish between this adventitial fat and the fat around the other vessels and glia cells during the first three months, all of it seems to be a waste product of myelin. This conception is further strengthened by a consideration of the fat which lies free without reference to either the glia cells or the vessels, a finding not expected if the fat has been taken from the vessels by the glia cells. Moreover, as a waste product, it is heaviest about the largest vessels instead of the smallest ones, from which it could be more easily stored if it were concerned in the building of myelin. But since it must be taken away by the vessels, it is meager or absent in those areas which have the best circulation, particularly a capillary one, as the cortex, cerebellum, basal ganglions and internal capsule, while it is pronounced in the regions of poor vascularity, as the centrum ovale, corpus callosum and lower part of the gyri. The lack of fat around the glia cells at the margins of the cortex may also be attributed to a vascularity which is that of the cortex, since the lower part of the sixth layer is a cortical fringe during the first few months of infancy. In the areas of poor vascularity, the glia cells apparently aid the fat in reaching the vessels, particularly the pseudopodia of the astrocytes, since with decrease of fat fewer glia cells have fat accumulations.

Rydberg considered the young astrocyte the cell around which most of the fat is precipitated. But, as can be seen in the photographs, the fat is scattered indiscriminately about both young and mature cells, although the young astrocyte is the chief cell numerically. He further thought that large naked nuclei in the white substance are forerunners of the astrocytes. The former may be observed in the photographs, but they are not more frequent than naked pseudopodia. Neither the nuclei nor the pseudopodia are in reality naked. They are thus represented because the softness of the young brain tissue bends and twists them out of the plane which would show the entire young astrocyte with its large nucleus, its subnuclear cytoplasm, fine processes and pseudopodia. The author also described lentil-shaped nuclei with processes and pseudopodia in the upper part of the centrum ovale as small spongioblasts, which are the origin of the microglia and oligodendroglia cells. These nuclei are found in both the upper and the lower part of the centrum ovale but are most prevalent in tissue seen long post

mortem. They are more distinct in the Cajal preparations, where the lentil-shaped nucleus is found to be either a thickened dark pseudopodium or massed cytoplasm running along beside or over a very pale clear round nucleus. The latter appears to be bent, while the pseudopodia and cytoplasm have experienced some postmortem change, much as in clasmatodendrosis. Rydberg likewise described strings of nuclei leading toward the centrum ovale from the ventricles, but by the Spielmeyer strain these are seen to be myelin sheaths.

Roback and Scherer believed that during myelinization a gliosis occurs in which the chief cell is also a large clear naked nucleus. The only naked nucleus is that of the medulloblast in the lower part of the centrum ovale, and this is small and dark. Moreover, gliosis is not a part of myelin formation in either the gray or the white substance. In the cortex, the myelin is formed among mature astrocytes, while the oligodendroglia cells increase chiefly as trabant cells. The astrocytes are less mature in the cerebellum, internal capsule and basal ganglions, but they are not undeveloped cells during the formation of myelin, as their general maturity at birth shows. The oligodendroglia cells are also few at the time of myelinization and at two years are less numerous than in the adult brain. The indifferent cells, or medulloblasts, of the centrum ovale are chiefly confined to the lower part, while the most numerous cells during the formation of myelin are the young astrocytes. However, the number of glia cells per field in the centrum ovale is greater at one year than during the first six months, as the number of oligodendroglia cells has greatly increased and that of the astrocytes has diminished but little. The rise in the oligodendroglia cells may be due to myelinization, as Penfield [14] held, since they multiply more rapidly from the third to the sixth month than in any other three month period, yet they begin to outnumber the astrocytes only after myelinization has apparently finished.

Although in this series the formation of myelin in the centrum ovale was complete at four months, its poor development in some of the younger infants suggests a more fluctuating termination. These variations occur in the upper part of the centrum ovale and in the gyri rather than in the lower part of the centrum ovale and are doubtless due to disease, although the effects of any one disease are not constant. A normal fluctuation, also, seems to take place, as the illustrations of Flechsig [15] show that regional as well as full myelinization varies slightly in brains of similar age. The development of the myelin in

14. Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 2.

15. Flechsig, P.: Anatomie des menschlichen Gehirns und Rückenmarks, Leipzig, Georg Thieme, 1920.

the cortex appears to progress in fairly regular fashion from the fourth to the ninth month, when the entire brain takes the myelin stain.

SUMMARY

In a study of the brains of forty-six infants varying in age from birth to 2 years, fat was found around the blood vessels and glia cells of the centrum ovale, corpus callosum and white substance of the lower part of the gyri in all of twenty-one infants less than 4 months of age.

The fat decreases with the formation of myelin and ceases at the age of 4 months, when myelinization in the white substance of the hemispheres is apparently complete.

This fat seems to be a waste product of myelin metabolism and occurs in areas of poor capillary circulation, while it is meager or absent in regions of good capillary circulation, such as the internal capsule, basal ganglions, cerebellum and cortex.

Perivascular fat is present in the large subcortical vessels from the first month to the end of two years of life, apparently as a metabolic product involving the myelin at the junction of the white and gray substances.

The formation of myelin is influenced somewhat by disease, since it varies not only at birth but during the entire period of its development.

The astrocytes are mature at the time of myelin formation in the cortex, between the fourth and ninth months of life. In the white substance of the cerebellum and in the centrum ovale, the chief cell during myelinization is a young astrocyte which has a large clear nucleus and subnuclear cytoplasm with fine processes and pseudopodia. The number of these cells is more than double that of the oligodendroglia cells in the centrum ovale during the first and second months, is equal to their number at six months and less than half their number at the end of two years, although the oligodendroglia cells are fewer than in the adult brain. The astrocytes of the centrum ovale apparently assist in transferring the waste fat of myelinization to the vessels by way of their pseudopodia.

DEVELOPMENT OF THE DIURNAL CYCLE OF LIVER FUNCTION IN NURSING RATS

A CHEMICAL AND HISTOLOGIC STUDY OF THE CONTENT OF GLYCOGEN AND OF BILE ACIDS IN THE LIVER CELL

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Forsgren [1] demonstrated a cyclic variation in the storage of glycogen and the secretion of the bile acids in the liver of the rabbit throughout the twenty-four hour period of the day. The glycogen content of the liver was found to be at its maximum during the night, at which time the production of bile acids reached its minimum; whereas during the daytime, particularly the afternoon, this relationship was reversed. The first period was called the assimilatory phase and the second period the dissimilatory phase of liver function. This rhythmic periodicity, moreover, has been proved to function to a great degree independently of nutritional factors. Forsgren employed the histochemical method of precipitating the glycogen in the liver cells by fixing the organ in absolute alcohol and staining it by Best's carmine method. The bile acids were stained by Mallory's method after the tissue had been treated with barium chloride and fixed in a 10 per cent solution of formaldehyde U. S. P. His observations on hepatic glycogen in rabbits were confirmed biochemically by Ågren and Wilander,[2] who found the maximal figure to be 13.1 Gm. of glycogen per hundred grams of fresh liver at 2 a. m. (8.6 Gm. of hepatic glycogen per kilogram of body weight); another peak in glycogen storage, though less pronounced, occurred at 4 p. m.; the minimal figure for hepatic glycogen was found to be 1.3 Gm. per hundred grams of liver (0.9 Gm. per kilogram of body weight) at 10 a. m. and 8 p. m.

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1. Forsgren, E.: (*a*) Ztschr. f. Zellforsch. u. mikr. Anat. **6**:547, 1927; (*b*) Skandinav. Arch. f. Physiol. **52**:137, 1928; **55**:144, 1929; (*c*) Ueber die Rhythmik der Leberfunktion, des Stoffwechsels und des Schlafes, Stockholm, Marcus, 1935.

2. Ågren, G., and Wilander, O., cited by Forsgren,[1c] p. 18.

Ågren, Wilander and Jorpes [3] repeated Forsgren's experiments on hepatic glycogen more extensively in mice and rats. Their findings agreed with his. In a series of 120 liberally fed white mice, they established a cyclic curve of hepatic glycogen with a peak of 2.5 mg. per gram of body weight at 2 a. m., and two minimums of 0.56 and 0.86 mg. per gram of body weight at 10 a. m. and 6 p. m., respectively, the figures representing the means for twelve groups of 10 animals each with determinations being made every two hours of the day. On the other hand, in a series of 120 rats starved for from twenty-four to forty-eight hours practically no hepatic glycogen was observable up to the fortieth hour if the organs were examined during the daytime; examined at night, however, even after more than forty hours of complete starvation, the livers of 10 animals showed a spontaneous rise in hepatic glycogen to a mean of 0.139 mg. per gram of body weight at 2 and 3 a. m. For the sake of comparison, the first curve obtained by these authors is reproduced in one of the accompanying charts (fig. 4).

Histochemically, Holmgren [4] in a series of twenty-four mice confirmed the alternating periodicity of the storage of glycogen and the secretion of bile acids during the day. A similar type of rhythmic alternation of the two substances in question was observed by Holmquist [5] in a series of 70 well nourished hooded rats over 3 months of age and weighing from 90 to about 200 Gm. In his curves, as in those established in rabbits, two peaks of hepatic glycogen occurred, one at 2 a. m. and the other at 2 p. m., corresponding to two minimums of bile acid secretion. The maximal average figure of hepatic glycogen was 4.6 Gm. per hundred grams of the fresh organ, or 2.45 mg. per gram of body weight. The minimal average figure for glycogen, found at 10 a. m. and 10 p. m., was 0.9 Gm. per hundred grams of liver, or 0.6 mg. per gram of body weight. The latter figures corresponded to a high bile acid content of the liver cells.

The only reports which disagree with the observations of the Swedish authors are those of Higgins, Bergson and Flock,[6] who maintained that the daily variations in hepatic glycogen are largely dependent on physiologic factors associated with the assimilation of food. However, they trained the rats to consume the food within two hours of the day, a decidedly unphysiologic procedure inasmuch as under normal physiologic conditions the ration is ingested gradually throughout the day, particularly at night. Thus, these authors produced an artificial increase in hepatic glycogen for from six to eight hours which, after a brief intervening period of decrease, was followed by another peak at

3. Ågren, G.; Wilander, O., and Jorpes, E.: Biochem. J. **25**: 777, 1931.
4. Holmgren, H.: Ztschr. f. mikr.-anat. Forsch. **24**:632, 1931.
5. Holmquist, A.: Ztschr. f. mikr.-anat. Forsch. **25**:30, 1931.
6. Higgins, G. M.; Bergson, G., and Flock, E.: Am. J. Physiol. **102**:637, 1932; **105**:177, 1933.

night without additional food. This secondary increase in hepatic glycogen occurring between 1 and 7 a. m. is, by the authors' admission, "probably correlated with the physiological process within the liver and perhaps with a rhythmic activity in the gastro-intestinal tract."

Since the experimental papers so far published are concerned exclusively with animals beyond the nursing period, the present investigation was undertaken chiefly for the purpose of determining whether or not a similar diurnal cycle exists in the liver of the young nursing rat, with special reference to the storage of glycogen and the secretion of bile acids.

MATERIAL

For the present study 168 rat pups were used, varying in age from 1 to 21 days, distributed as follows:

Group 1, from 1 to 7 days old (first week)	48
Group 2, from 8 to 14 days old (second week)	44
Group 3, from 15 to 21 days old (third week)	76
Total	168

The oldest animals of the third group were taken just prior to weaning.

METHODS OF INVESTIGATION

All animals used in the present investigation belonged to the same highly homogeneous breed. In many experiments from 4 to 14 rat pups of the same litter were used. The nursing mothers were continuously fed on an adequate standard diet, high in calories, and kept with their litters in cages fully exposed to the natural daily variations of light and darkness. The experiments were all conducted during the winter months (from the middle of December to the end of March), and no attempt was made to select any particular age group at any time throughout the experiments. Special attention was paid to whether or not the rats were nursing at the time of observation. All hours of the twenty-four hour period are represented in the experiments. As a rule two animals were used at a time for each two hour period, being weighed separately and then killed by a gentle blow on the head. The abdomen was opened at once, and the condition of the stomach (full, medium or empty) was noted. The liver was quickly removed, weighed and frozen in carbon dioxide snow for chemical analysis. Before freezing, a small portion of the liver was fixed either in absolute alcohol for the histologic demonstration of glycogen or in 3 per cent barium chloride solution for the staining of bile acids by Mallory's method (Forsgren [1a]).

For the chemical determination of hepatic glycogen the frozen tissue was weighed, the average amount of the material used for actual analysis being 0.46 Gm. (ranging between 0.09 and 1 Gm.). After Pflueger's [7] method as modified by Good, Kramer and Somogyi,[8] the tissue was boiled in a 30 per cent potassium hydroxide solution, precipitated with 95 per cent alcohol, centrifugated, drained and finally hydrolyzed with a sixth-tenths normal hydrochloric acid solution for at least two hours. The dextrose was determined according to the method of Somogyi, Shaffer and Hartmann,[9] the figures being multiplied by 0.927 in calcu-

7. Pflueger, E.: Arch. f. d. ges. Physiol. **102**:305, 1904.
8. Good, C. A.; Kramer, H., and Somogyi, M.: J. Biol. Chem. **100**:485, 1933.
9. Somogyi, M.: J. Biol. Chem. **70**:599, 1926.

lating the values for glycogen (Nerking[10]). The precipitated and centrifugated glycogen, covered with the alcohol-hydroxide mixture, may be kept in the refrigerator for about twenty hours without any changes in the values, as pointed out by van Creveld[11] in his method for the determination of glycogen in the blood.

PRESENTATION OF RESULTS

Hepatic Glycogen.—1. Increase in Liver Weight in Relation to Body Weight in General. During the first three weeks of life the body

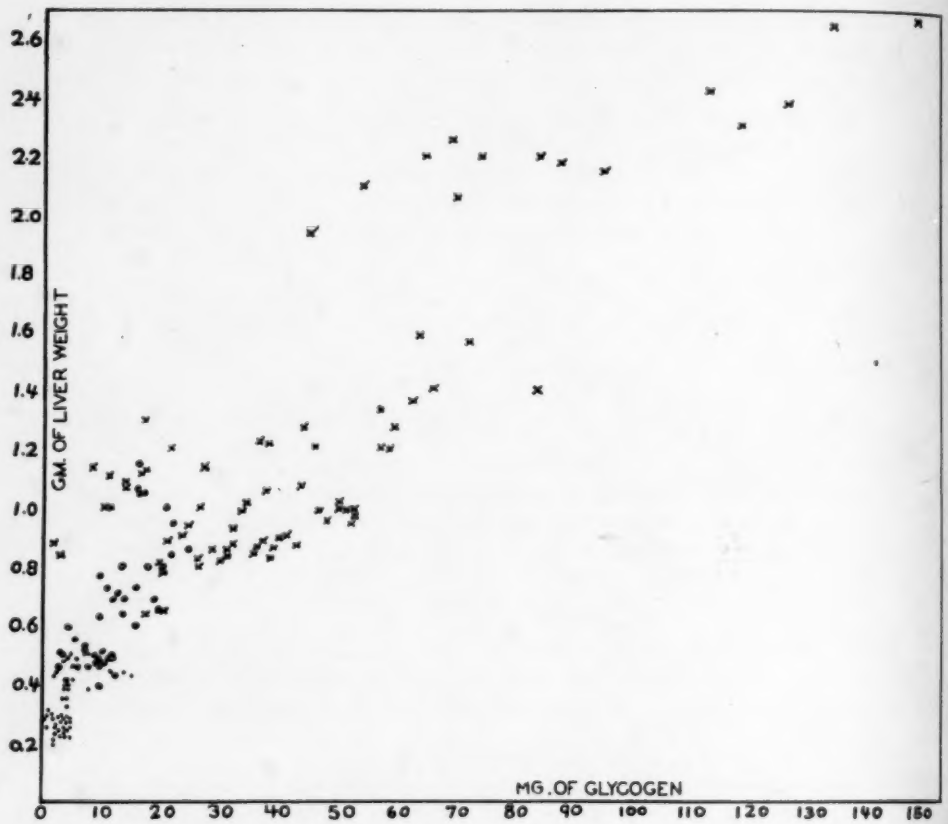


Fig. 1.—Scatter diagram of actual values for hepatic glycogen (in milligrams), plotted against the weight of fresh liver (in grams). The values obtained from animals during the first week of postnatal life are represented by small dots; those obtained during the second week, by small circles, and those during the third week by crosses.

weight of the rat increases rapidly. During the first week it varies from 5 to 18 Gm., during the second week from 13 to 34 Gm. and during the

10. Nerking, F.: Arch. f. d. ges. Physiol. **85**:320, 1901.
11. van Creveld, S.: Arch. Dis. Childhood **9**:9, 1934.

third week from 23 to 69 Gm. The variations in the weight of the liver are from 0.19 to 0.50 Gm. during the first week, from 0.39 to 1.15 Gm. during the second and from 0.64 to 2.67 Gm. during the third week. In relation to body weight, the weight of the liver varies during the first week between 2.4 and 4.8 per cent (mean, 3.4 per cent), during the second week between 2.1 and 4.2 per cent (mean, 2.8 per cent) and during the third week between 2.8 and 4.9 per cent (mean, 3.4 per cent). Thus, in the present experiment, there was a slight retardation in the relative growth of the liver during the second week as compared with that of the first and third weeks. In the older but still rapidly growing rats used by Holmquist [5] the mean relative weight of the liver was 4.7 per cent of the body weight. The average value for fully grown rats is approximately 3 per cent.

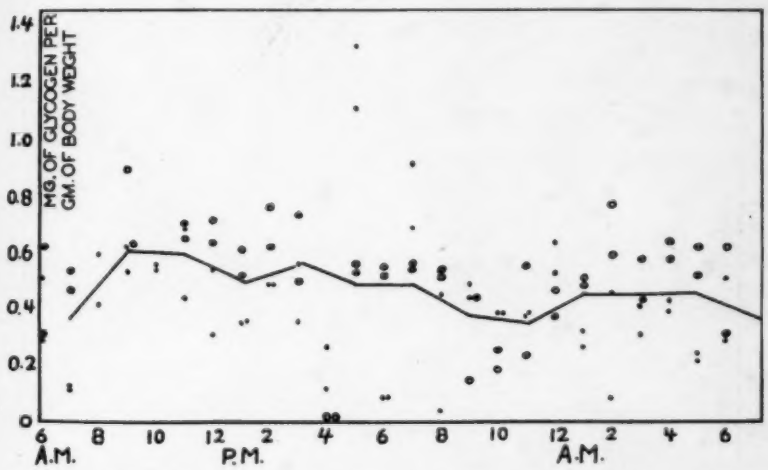


Fig. 2.—Scatter diagram of values for hepatic glycogen (in milligrams per gram of body weight) during the first two weeks, plotted against the two hour intervals of the twenty-four hour period, computed from the actual amounts shown in figure 1. The values calculated during the first week of life are represented by small dots; those during the second week, by small circles. The continuous line connects the mean values at two hour periods.

2. Increase in Glycogen Content of the Liver in General. The actual values for hepatic glycogen as related to the weight of the fresh liver of the rat pup are given in figure 1. The increase in age and in the weight of the liver is accompanied by an increase in the glycogen content of the organ. However, it should be noted that, while the figures obtained during the first two weeks of life fall within rather well defined and relatively restricted areas, without much scattering, those obtained during the third week show a high degree of scattering, with a distinct shift to the areas for higher glycogen values. The differ-

ence between the two periods becomes even more clearly accentuated when the actual values for hepatic glycogen are translated into percental figures in relation to the body weight. Calculated in milligrams of glycogen per gram of body weight, hepatic glycogen rarely rises above the 0.75 mg. level in the first two weeks (fig. 2), whereas it seldom drops below that level in the third week (fig. 3). During the first week, hepatic glycogen varies between 0.034 and 1.308 mg. per gram of body weight, during the second week between 0.011 and 0.880 mg. and during

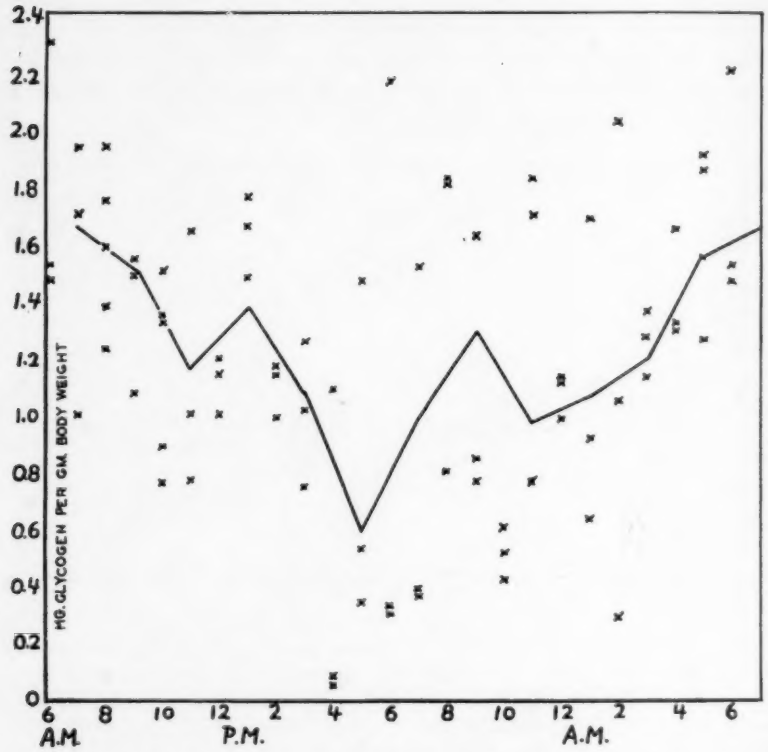


Fig. 3.—Scatter diagram of values for hepatic glycogen (in milligrams per gram of body weight) during the third week, plotted against the two hour intervals. All values are represented by crosses. The continuous line connects the mean values at two hour periods.

the third week between 0.051 and 2.298 mg. per gram of body weight, the average values for these weeks being 0.403, 0.504 and 1.198 mg. per gram of body weight, respectively. The corresponding figures for glycogen in grams per hundred grams of fresh liver range from 0.11 to 3.54 Gm. during the first week of life, from 0.05 to 3 Gm. during the second week and from 0.17 to 5.97 Gm. during the third week, the average values being 1.22, 1.79 and 3.50 Gm., respectively.

It is clear, therefore, that a spectacular rise in the amount of glycogen stored in the liver occurs during the third week of postnatal life. This rise in the glycogen content of the liver during the third week is at least twice the initial amount present in the younger animal. The rise appears rather suddenly at the end of the second week, since at the age of 14 days, 4 of 6 animals show storage of from 0.88 to 1.95 mg. of glycogen per gram of body weight whereas up to the age of 13 days the average level is below 0.75 mg. per gram of body weight. A certain percentage of animals at the age of 14 days, therefore, as judged from the ability of the liver to store glycogen, belong definitely to the older age group of the third week.

3. Glycogen Content of the Liver at Hourly Intervals. The results obtained on calculating the hourly values of the glycogen content of the

TABLE 1.—*Mean Values and Average Deviations of Hepatic Glycogen in Nursing Rats at Two Hour Intervals*

Hours	First and Second Weeks (Groups of 8 Animals)	Third Week (Groups of 6 Animals)
6- 7 a.m.	0.364 (±0.161)*	1.658 (±0.323)*
8- 9 a.m.	0.606 (±0.100)†	1.499 (±0.209)#
10-11 a.m.	0.585 (±0.081)†	1.158 (±0.297)#
12- 1 p.m.	0.494 (±0.123)	1.375 (±0.297)
2- 3 p.m.	0.555 (±0.104)	1.072 (±0.149)
4- 5 p.m.	0.483 (±0.385)	0.596 (±0.457)
6- 7 p.m.	0.484 (±0.202)	0.881 (±0.642)
8- 9 p.m.	0.374 (±0.144)	1.278 (±0.471)
10-11 p.m.	0.340 (±0.060)	0.975 (±0.528)
12- 1 a.m.	0.439 (±0.095)	1.062 (±0.247)
2- 3 a.m.	0.445 (±0.142)	1.190 (±0.368)
4- 5 a.m.	0.444 (±0.133)	1.549 (±0.257)

* All values are expressed in milligrams per gram of body weight.
† Six animals are represented.
# Eight animals are represented.

liver in milligrams per gram of body weight for all animals under the age of 14 days are grouped together because of their decided similarity (fig. 2), while those obtained for the animals older than 14 days form a separate group (fig. 3). Mean values have been calculated from the data obtained in each age group, in groups of from 6 to 8 animals, at two hour intervals throughout the day (table 1).

During the first two weeks of life the mean values, with the exception of those obtained at 4 and 5 p. m., reveal little average deviation, and the curve of the mean values (fig. 2) ranges only from 0.4 to 0.6 mg. per gram of body weight, exhibiting but a slight peak at 10 a. m. and a moderate minimum at 10 p. m. Hence it can be definitely stated that practically no rhythmic change occurs in the amount of glycogen stored in the liver during the entire day in rats younger than 14 days. But in older rats the picture differs decidedly. Between midnight and 3 p. m., the average deviation from the mean is within normal limits, but between 4 and 11 p. m. the deviations are so great as to make questionable the practicability of calculating means during this period. At any

rate, late afternoon and evening (4 p. m. to 2 a. m.) constitute the periods during which hepatic glycogen drops below the level of 0.75 mg. per gram of body weight (fig. 3). The curve of mean values reaches its lowest level at from 4 to 5 p. m., whereas the values obtained during the early morning hours and the forenoon (from 2 a. m. to 1 p. m.) frequently surpass 1.1 mg. per gram of body weight, the curve of mean values reaching the peak at 6 a. m. A similar curve is obtained when glycogen is plotted in grams per hundred grams of fresh liver against the twenty-four hour period.

These figures demonstrate the unmistakable tendency in older rat pups (from 14 to 21 days of age) to manifest rhythmic changes in

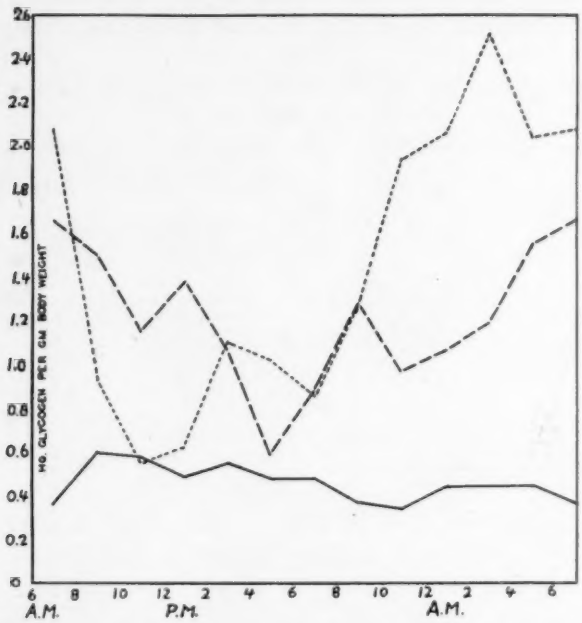


Fig. 4.—Comparative chart showing the diurnal variations in mean values for hepatic glycogen (in milligrams per gram of body weight) plotted against two hour intervals. The continuous line indicates the mean curve for rat pups during the first and second weeks of life; the broken line, for those during the third week; the dotted line from the work of Agren, Wilander and Jorpes [3] is given for comparison and represents a similar mean curve for 120 liberally fed adult mice.

hepatic glycogen during the twenty-four hour period, the high storage occurring during the early morning and the decline during the late afternoon. This tendency toward development of a distinct rhythm in liver function taking place during the third week of life confirms in its general pattern the observation by other investigators of a similar though more pronounced rhythm in older animals (fig. 4).

The nutritional conditions of the rat pups used in this investigation require a brief analysis with special reference to the daily cycle. According to the findings summarized in table 2, the nursing activity in both groups of animals is at its minimum from 4 to 11 p. m. and at its maximum between midnight and 7 a. m. This observation agrees with that of Higgins and his co-workers, who found the highest feeding activities in adult rats during the night. In spite of these facts, the stomachs of the rat pups were found to be filled with milk clots at practically every hour of the day, the exceptions in both groups being only in the period between 4 and 11 p. m. That is to say, the nutritional conditions were quite similar for both groups, while the hepatic glycogen curves reveal a striking difference, the decline between 2 and 8 p. m. being definite in the older group but practically absent in the younger

TABLE 2.—*Nutritional Conditions of Experimental Animals*

Age, Weeks	Hours	Rats Nursing	Rats Not Nursing	Rats Whose Stomachs Were		
				Full	Medium	Empty
1-2	8 a.m. - 3 p.m.	9	7	14	2	0
	4 p.m. - 11 p.m.	0	19	21	2	5
	12 a.m. - 7 a.m.	18	8	23	3	0
3	8 a.m. - 3 p.m.	4	10	12	2	0
	4 p.m. - 11 p.m.	7	17	19	3	2
	12 a.m. - 7 a.m.	17	7	21	3	0

group. In individual cases, moreover, no regular correlation was observable between the nutritional factors and the glycogen content of the liver. It seems, then, that the rhythmic cycle of the storage of glycogen in the liver, present only in older animals, cannot be explained solely on the basis of nutritional conditions.

Bile Acids.—Bile acids in the liver cells were estimated qualitatively by the histochemical method already described (Forsgren). In each instance the amount of bile acids was judged from the character of the cytoplasmic pattern. Thus the liver cell which was closely packed with fine or coarse granules stained red or reddish violet was recorded as having the maximal amount of bile acids, and the cell relatively scant in such granules because of the empty spaces presumably left by the dissolution of glycogen was classed as having the minimal amount of the acids. Hence, the classification is more or less arbitrary, but for the sake of convenience the graded amounts of bile acids in the liver cells were designated by one, two and three plus signs (minimal, moderate

and maximal) according to the amounts of detectable granules (fig. 5). The estimation of bile acids was made independently of the results of analyses for glycogen.

The results from the estimation of bile acids are presented in table 3. Examination of the data reveals that while in the group of rat pups less than 2 weeks old a small number of animals showed, mostly during the forenoon hours, a minimum amount of bile acids in their liver cells, the great majority produced a maximal or moderate amount at all hours of the twenty-four hour period. Also it is to be noted that no animal's liver cells showed the minimal quantity of bile acids during the afternoon and throughout the night. During these hours, in all animals the bile acid content of the liver cell was relatively high.

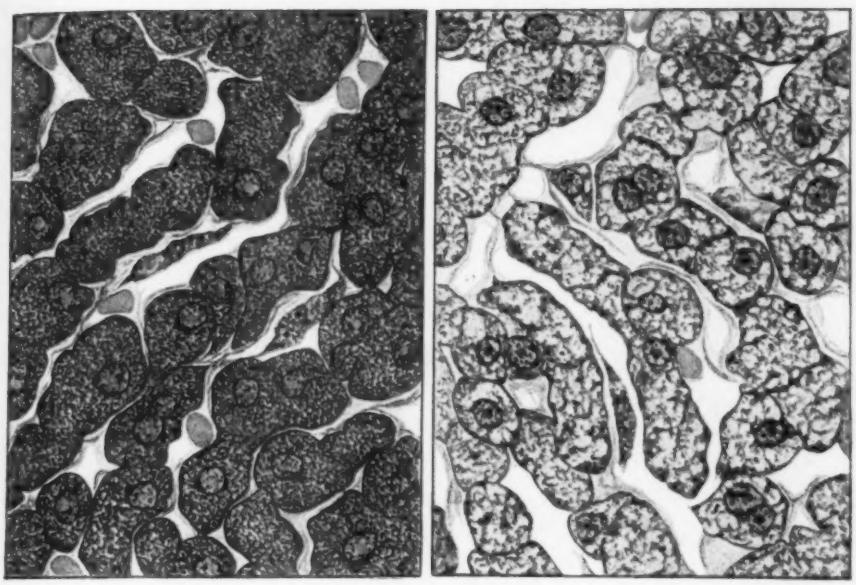
TABLE 3.—*Graded Classification of Bile Acid Content of Liver Cells at Two-Hour Intervals* *

Hours	Rats Showing Given Grade in First and Second Weeks			Rats Showing Given Grade in Third Week		
	+	++	+++	+	++	+++
6- 7 a.m.	5	1	4	2	4	0
8- 9 a.m.	2	3	3	4	3	0
10-11 a.m.	2	3	5	0	6	0
12- 1 p.m.	2	2	4	2	4	0
2- 3 p.m.	0	4	2	2	4	1
4- 5 p.m.	0	5	2	1	2	3
6- 7 p.m.	0	4	4	2	1	3
8- 9 p.m.	0	2	5	4	0	6
10-11 p.m.	0	7	1	5	1	2
12- 1 a.m.	0	2	4	0	4	2
2- 3 a.m.	0	3	7	5	0	0
4- 5 a.m.	0	4	8	5	1	0

* The table includes all animals the livers of which were sectioned and studied histologically, exceeding in number the livers used for chemical analysis.

The picture, however, was quite different in the older group of animals in their third week of postnatal life. The majority of them showed the minimal or moderate amount of bile acids, while the maximal amount of the acids was recorded only during the late afternoon and evening (from 3 p. m. to midnight). It is significant that in no animals did the bile acid content of the liver cells reach its maximum during the early morning and forenoon (from 2 a. m. to 1 p. m.).

The foregoing observation correlates remarkably with the results of the quantitative analyses for hepatic glycogen presented in the preceding section. Since both glycogen and bile acids appear to be metabolized in the parenchymal cells of the liver, these two substances have a reciprocal relationship within the cells. During the first two weeks of life, hepatic glycogen is found to be rather continuously on a low level and bile acids correspondingly on a high level. In the third week, during certain hours of the day, particularly during the early morning and forenoon, glycogen appears to be the substance abundantly stored in the liver cells, bile acids playing only a minor role. During



EXPLANATION OF FIGURE 5

The microscopic appearance of liver cells of nursing rats at 3 a. m.: right, liver of a 7 day old rat; left, liver of a 21 day old rat. Note the accumulation of precipitated and stained bile acids (represented by closely packed, deep violet granules) in the cells of the younger animal, in contrast with the presence of relatively few bile acid granules and numerous vacuoles (due to dissolution of glycogen) in the cells of the older animal. The glycogen content of the liver in the younger rat was 1.38 Gm. per hundred grams of liver, or 0.402 mg. per gram of body weight; in the older rat the glycogen content of the liver was 4.01 Gm. per hundred grams of liver, or 1.366 mg. per gram of body weight. (Barium chloride and formaldehyde fixation; paraffin embedding; Mallory's aniline blue-orange G stain; camera lucida drawing, with use of oil immerson and × 10 ocular.)

the other period of the day, however, particularly the late afternoon and evening, bile acids are predominantly secreted by the liver cells, glycogen being deposited only in negligible quantities.

COMMENT ON RESULTS

Determinations of hepatic glycogen in the fetus and in new-born animals have been made by Pflueger [7] and more recently by Sake [12] and Wertheimer.[13] In calves, guinea pigs, rats and rabbits, these authors found the amount of hepatic glycogen in immature animals to vary greatly, the actual values ranging between a trace and 6.5 Gm. per hundred grams of liver. None of them stated the exact hours of the day at which the experimental animals were killed. In 11 rat fetuses of the same litter at midpregnancy (from 4 to 5 Gm. in weight) Sake reported figures obtained by an indirect method as varying from about 1 to 3.4 Gm. per hundred grams of liver, closely corresponding to our results obtained in rat pups during the first two weeks of life. Figures published by Sake for guinea pig and rabbit fetuses and by Burghard and Paffrath [14] and Hertz [15] for human fetuses, all obtained at various stages of prenatal development, indicate a rather steady rise in the glycogen content of the liver from about 0.5 Gm. per hundred grams of liver in the early stages to between 2 and 5 Gm. at or near the end of pregnancy. These values are obviously not comparable because of the fact that the fetuses of different species are born at different stages of development (e. g., calves, guinea pigs, rabbits and rats represent decreasing degrees of maturation at birth in the order given).

As yet no experiments have been published on the influence of the diurnal cycle on the glycogen content of the liver in the young growing animal. Sake [12] and Wertheimer,[13] however, studied the changes in the glycogen content of the liver in fetal and newly born guinea pigs and rats under various experimental conditions. For example, they subjected pregnant and new-born animals to the various stimuli known to mobilize glycogen from the liver, such as anoxybiosis, cold and injections of epinephrine. While these agents render the maternal organism, excepting the uterus, practically glycogen free, they fail to inhibit in the fetuses and the new-born animals the steadfast preservation of glycogen in the liver, muscles and the rest of the body. The effect is found to be more pronounced in rats than in guinea pigs, the latter being more mature at birth. The glycogen reserve of the fetus and of the new-born animal, therefore, appears to be decidedly unaffected by the regulatory influences which govern the glycogen content of mature organisms.

12. Sake, A.: Ztschr. f. Kinderh. **45**:93, 1928.
13. Wertheimer, E.: Arch. f. d. ges. Physiol. **223**:619, 1930.
14. Burghard, E., and Paffrath, H.: Ztschr. f. Kinderh. **45**:68, 1928.
15. Hertz, W.: Ztschr. f. Kinderh. **55**:410, 1933.

"The glycogen reserves," to quote Wertheimer, "cannot be mobilized by external stimuli (in young animals) as they can in adult animals. Only gradually, with the cessation of rapid growth tendency, does the animal develop the power to accommodate to the regulations taking place in the organism as a whole." The cellular glycogen-mobilizing factors in immature animals respond very poorly to irritations of the autonomous nervous system that in adult organisms readily lead to glycogenolysis.

The present experiments further confirm this lack of response in young growing animals. Here, the external stimulus applied is the rhythmic cycle furnished by the change of day and night in the course of twenty-four hours. In adult organisms this stimulus, according to the review published by Jores,[16] is transferred from the sensory organs to the liver and other tissues through hormonal action and by autonomic pathways, the epinephrine output and sympathetic tonus being high during the day but low at night, and the posterior pituitary secretion and parasympathetic tonus being low during the day but high at night. For some reason, during the first two weeks of the rat's life the external stimulus fails to reach the local glycogen-mobilizing system in tissue cells, particularly in the liver, becoming effective only on or about the fourteenth day and thereafter. Therefore, in young rats no relationship exists between the hepatic glycogen and diurnal changes or any other external stimuli, the level of glycogen being consistently low, while that of bile acids is high. But during the week just prior to weaning the glycogen of the liver does become amenable to the influence of the diurnal changes in atmospheric and cosmic conditions, its content being rhythmically changed from a rather high level to a temporary low one, whereas the fluctuations in bile acids tend to assume the opposite course. This rhythmic periodicity of liver function is definitely established in adult life.

Finally, the question arises as to how the rhythmic metabolism of glycogen and bile acids develops in human subjects. Forsgren and his co-workers demonstrated the cyclic changes in liver function in human adults by indirect methods—for example by estimating the metabolism of water, nitrogen and urobilin as well as by study of the body temperature, sleep and other phases of metabolism. In a pregnant woman suffering from afebrile ulcerative tuberculosis of the lungs, Forsgren and Schnell[17] had an opportunity to study the liver function during the last trimester. They reported "neutralization" of rhythmic changes in metabolism and body temperature, "probably as a result of the metabolism in the uterus and fetus which are not, as far as we know, subjected to the daily variations characteristic of the liver functioning. The

16. Jores, A.: Ergebn. d. inn. Med. u. Kinderh. **48**:574, 1935.
17. Forsgren, E., and Schnell, R.: Acta med. Scandinav. **82**:155, 1934.

fetus and later the new-born child presented a fairly continuous functioning with no division into active day and passive night, a circumstance which apparently contributed to evening out the rhythmic variations of the mother's metabolism." The authors did not discuss in detail how this observation may be explained.

In adult subjects, Forsgren reported, the daily body temperature curve has waves parallel to those of the dissimilatory or bile acids secreting function of the liver and consequently constitutes a mirror image of the assimilatory or glycogen-storing function of the organ. Holmquist directly demonstrated this parallelism in adult rats in which the daily body temperature and the daily secretion of bile acids produced rising curves during the period of minimal storage of glycogen, and vice versa. From such a parallelism one infers that in healthy infants a development of rhythmic liver functioning similar to that found in nursing rats might be reflected by the well known development of the diurnal cycle of body temperature at different stages of growth. According to the careful investigations of Jundell [18] and Gofferjé,[19] the twenty-four hour temperature curve of the normal infant during the neonatal period is practically a straight line, with a maximum deviation of only ± 0.09 C. (0.16 F.). Even in babies from 1 to 2 months old there is but a slight deviation of temperature during the day (± 0.25 to 0.30 C. [0.45 to 0.54 F.]). These observations constitute the basis for the phenomenon known as monothermia of the young infant. A definite rhythm in body temperature, with its peak during the day, particularly in the afternoon, and a depression at night (± 0.57 C. [1.02 F.]), develops at the age of from 2.5 to 6 months. A more pronounced decline in body temperature during the night developing at the age of from 2 to 5 years gives the temperature curve an even greater swing in older children and in adults (±0.95 and 0.83 C. [1.71 and 1.49 F.], respectively). If there is a parallelism between body temperature and liver function in infants, these observations may indicate a virtual lack of rhythmic periodicity of liver function in young infants under 3 months of age, the distinct diurnal cycle developing only at the turn of the first half year and the very definite one during childhood, which persists throughout life. This inference is reasonably supported by the results of the present investigation in young growing rats. During the first two weeks of the rat's life (corresponding in human beings to the period covered by the last months of intra-uterine up to the first two months of extra-uterine life) no distinct cyclic change in liver function occurs, while during the third week (corresponding to the period covered by

18. Jundell, J.: Jahrb. f. Kinderh. **59**:521, 1904.
19. Gofferjé, F.: Jahrb. f. Kinderh. **68**:131, 1908.

about the third to the ninth month of human postnatal life) a definite cycle, as judged by the liver cell's activity in storing glycogen and secreting bile acids, begins to develop.

SUMMARY

In 168 young growing rats from 1 to 21 days old chemical and histologic analyses were made with a view to quantitative estimation of hepatic glycogen and microchemical demonstration of bile acids in hepatic cells at hourly intervals during the twenty-four hour period.

Hepatic glycogen and bile acids demonstrate a mutually reciprocal relationship in that when the one is present abundantly in the liver cells the other is proportionately reduced in amount.

Hourly analyses for hepatic glycogen and bile acids during the day demonstrate the development of a diurnal cycle in their production (as was determined by other investigators in adult subjects) during the first weeks of the animal's life.

In rat pups up to the age of 13 days both substances are more or less uniform in quantity during the twenty-four hour period, the values for hepatic glycogen being persistently low and those for bile acids relatively high. At that age, no definite diurnal cycle of liver function is demonstrable.

In rat pups from 14 to 21 days of age, however, liver function shows a definite diurnal cycle in glycogen storage and bile acid secretion. The glycogen content (reaching a much higher level than during the first two weeks of life) has its maximum during the early morning and most of the forenoon, while the minimum occurs in the late afternoon. The curve of bile acids is a mirror image of that of glycogen.

In both groups of rat pups, the curves of liver function are to a large extent independent of the nursing conditions of the animals.

The results obtained by the present experiments add further proof for the existence of a diurnal cycle in liver function paralleling similar variations in body temperature and other metabolic functions as demonstrated by previous investigations.

Case Reports

THROMBOTIC OCCLUSION OF SUPERIOR VENA CAVA AND ITS TRIBUTARIES, ASSOCIATED WITH ESTABLISHED COLLATERAL CIRCULATION

F. J. L. Blasingame, M.D., Galveston, Texas

Instances of complete thrombotic occlusion of the superior vena cava and innominate veins, associated with partial occlusion of the subclavian and internal jugular veins in man, with establishment of adequate collateral circulation, are extremely rare. Ochsner and Dixon [1] in an exhaustive review of the literature were able to find only 120 instances of thrombosis of the superior vena cava, some of which were not definitely proved to be such. They gave a complete bibliography and reported 2 more cases. In the subsequent literature, I have been able to find an additional instance recorded by Szour and Berman.[2] I here report a case of extensive thrombosis of this description observed in a cadaver. Since the lives of persons thus affected and the signs and symptoms exhibited are dependent on the establishment of compensatory circulation, advantage has been taken of the opportunity to examine the various routes by which collateral circulation was established in this case.

REPORT OF CASE

The subject was a white man aged 93 years. The record gave cardiorenal disease as the cause of death. No clinical history was obtainable.

The body was muscular and well nourished and weighed about 180 pounds (81.7 Kg.). Surface examination gave no evidence of trauma or of disorder in the venous system.

Dissection was begun in the usual manner in the regular course of gross anatomic examination, by students dissecting the extremities, the head and neck, and part of the thorax. The remainder of the dissection was made by me.

In the description only significant facts are included, emphasis being placed on the veins. The description may be more easily followed by referring to the accompanying diagram (fig. 1).

In the extremities the veins were larger than normal, especially those of the lower limbs—e. g., the left femoral vein measured 24 mm. in diameter just below the inguinal ligament. In the upper extremities, while most of the veins were somewhat enlarged, the upper parts of the axillary veins were rather small, but unusually large lateral thoracic veins connected the venous return from the upper extremities with the intercostal veins.

The subclavian veins were smaller than usual, being partially occluded by canalized thrombi in their lower portions and completely occluded in their upper portions; these veins received from above the external jugular veins, which

From the Laboratory of Anatomy, University of Texas.

1. Ochsner, A., and Dixon, L. L.: J. Thoracic Surg. **5**:641, 1936.

2. Szour, M., and Berman, R.: Rev. de la tuberc. **2**:1068, 1936.

in turn were connected with enlarged transverse scapular and transverse cervical veins. The transverse scapular and transverse cervical veins established unusual connections with the upper intercostal veins, near the posterior extent of the intercostal spaces.

Dissection of the head and neck showed large jugular and thyroid veins, with the right internal jugular vein being completely thrombosed near its lower

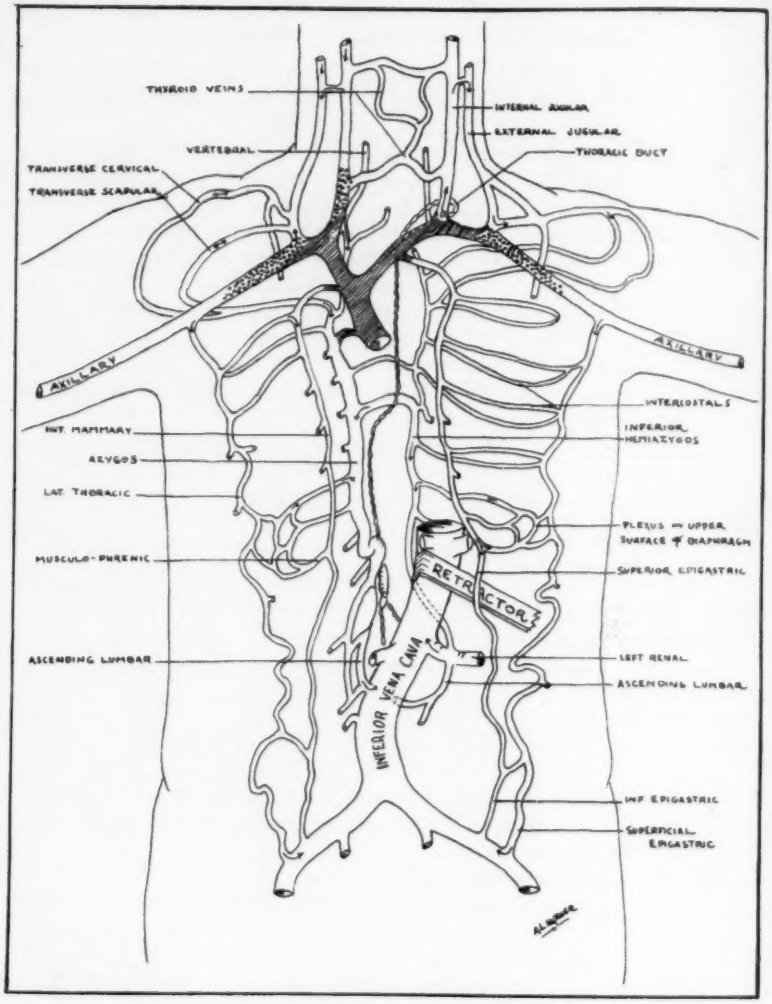


Fig. 1.—Diagram of veins, showing extent of thrombosis and routes of collateral circulation. The hatching indicates complete occlusion, while the small circles in certain veins show thrombosis with canalization. The arrows indicate the probable direction of blood flow.

end and partially occluded in its lower third. The left internal jugular vein was patent throughout; it received in its lower lateral aspect the thoracic duct and several small tributaries and then joined below the completely occluded left

subclavian and innominate veins. Hence, the blood and added lymph had to flow upward in the lower parts of the internal jugular veins and over into the external jugular veins, then down the latter veins through their connections with the transverse scapular and transverse cervical veins, which, as has already been pointed out, were connected below with the upper intercostal veins.

In dissection of the thorax, it was found that a cylinder of fibrous tissue, showing slight central calcification and measuring from 0.5 to 1 cm. in diameter, had replaced the superior vena cava and was slightly adherent posteromedially

Fig. 2.—Photograph showing thrombosed vessels. Note the marked reduction in size of the superior vena cava and in the innominate, internal jugular, and subclavian veins.

to the outer portion of the trachea. This fibrous remnant of the superior vena cava became continuous below with the right atrium and above with the thrombosed innominate veins. The other mediastinal structures were normal and showed no tendency to encroach on the great veins. The areolar tissue was loose and flaky and dissected away from the veins easily.

The thorax and abdomen showed marked dilatation of the collateral venous circulation dorsally and ventrally. Dorsally, the enlarged intercostal veins on

the right side drained into the azygos vein, while those on the left went into the superior and inferior hemiazygos vein. The azygos and hemiazygos veins were connected across the midline posteriorly by several transverse channels and in the lower part of the posterior mediastinum received large tributaries from the esophageal plexus and also tributaries from large plexuses on the upper surface of the diaphragm. The azygos vein continued inferiorly as the right ascending lumbar vein, which in turn connected with the inferior vena cava. The inferior hemiazygos vein connected below with the left renal vein; the left renal vein, in addition to its usual opening into the inferior vena cava, was connected by an ascending lumbar vein to the inferior vena cava.

As was pointed out, the lateral thoracic veins made large communications with the intercostal veins along the anterolateral aspect of the wall of the chest. The lateral thoracic veins also communicated with a large plexus on the upper surface of the diaphragm and then continued inferiorly in a very tortuous, varicose manner to connect with the upper end of the superficial epigastric veins, which, after receiving many subcutaneous tributaries from the abdominal wall, ended below in the femoral veins.

Although the upper parts of the internal mammary veins were thrombosed for a short distance, the remainder of each of these was quite enlarged and drained mainly into dilated intercostal veins. Inferiorly, the internal mammary veins divided on the diaphragm into enlarged plexiform musculophrenic veins and normal superior epigastric veins. The plexus on the diaphragm, as was mentioned, drained dorsally into the azygos and inferior hemiazygos veins. The superior epigastric vein became continuous with the inferior epigastric vein which emptied into the external iliac vein. Passing either lateral to or through the rectus abdominis and the anterior layer of its sheath were anastomoses between the superior and inferior epigastric veins and the enlarged lateral thoracic and superficial epigastric veins. Decidedly more of the blood passed from above downward in the lateral thoracic and superficial epigastric veins than through the superior and inferior epigastric veins.

The veins of the portal system showed no enlargement. There was no enlargement of the thoracic duct or any other lymph channel.

The heart was normal in size except that there was slight dilatation of the right atrium. The tricuspid and mitral valve leaflets were thickened, and these valves were moderately incompetent. The pulmonary and aortic valves appeared normal. The endocardium on the walls of the cavities was everywhere smooth and glistening. The arteries showed only slight sclerosis. The kidneys showed no significant pathologic changes. These findings in the heart and kidneys failed to substantiate the clinical diagnosis of caridorenal disease as the cause of death.

There was no skull fracture; but a recent subdural hematoma on each side covered and compressed most of the superolateral aspects of each cerebral hemisphere. This intracranial hemorrhage probably was the cause of death.

SUMMARY

Because of the chronic character of the thrombosis and the extent of dilatation of collateral vessels, together with the recent intracranial hemorrhage, it may be assumed that the thrombosis had existed for a relatively long period of time and that adequate collateral circulation had become established. In the absence of any history on the case the cause of this extensive thrombosis remains unknown. The case illustrates again the adaptability of the venous system to alterations in pressure.

TUBERCULOUS SEPSIS WITH A MYELOBLASTIC BLOOD PICTURE

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The patient, a 29 year old unmarried white woman, was admitted to the Beth Israel Hospital to the service of the late Dr. Marcus A. Rothschild on March 11, 1933, complaining of vomiting, fever and headaches, which had been present five weeks. The illness began with a bitter taste in the mouth, followed by an eruption on the gums with discharge of blood-tinged pus. She vomited almost everything she ate. The temperature at the onset was 103 F., and the fever continued almost without fluctuation until her admission to the hospital. The lesions of the mouth improved prior to admission, but the bitter taste persisted. There had been no cough, expectoration of sputum or hemoptysis until twenty-four hours before admission, when she began to have pain in the left side of the chest and slight cough. Within the last two months she had lost from 30 to 40 pounds (13.5 to 18 Kg.). Two months before admission, an eruption appeared over both feet, with moderate edema around the ankles. This persisted until entrance into the hospital.

The family history was entirely without bearing on the case. There was no history of tuberculosis. The patient had typhoid fever fourteen years prior to this time. Three years before, in Beth Israel Hospital, she had tuberculosis of the lower dorsal region of the spine, for which a closed jacket was applied. At that time there was also roentgen evidence of exudative phthisical changes within the apexes of both lungs.

On admission the patient was acutely ill. The temperature was 103.4 F., the pulse rate 124, the respirations 30. The skin was pale, with frequent unilateral flushing of the face. There was no ulceration of the buccal membrane. There was a small reddish area on the pharynx, not definitely ulcerated. The lymph nodes were normal. There were dulness and diminished breath sounds over the lower lobe of the left lung and a few râles with dulness and diminished breath sounds over both apexes. The abdomen disclosed no abnormality. Over the lower third of the legs there were discrete purplish papules, crusted and excoriated. The reflexes were normal.

An x-ray picture of the chest four days after admission showed tuberculous inflammation at the apexes of both lungs and a small effusion in the left costophrenic space. The outstanding laboratory finding was the blood picture. The total white blood cell count varied between 3,200 and 1,250. The differential count showed about 40 to 55 per cent myeloblasts and promyelocytes, and about 35 per cent lymphocytes. The percentage of mature nucleated white cells present was small. The platelets numbered 80,000; the red blood cells, about 2,000,000; the hemoglobin was about 50 per cent. The sedimentation rate of the erythrocytes on two separate occasions was 95 and 85 mm. (forty-five minutes), respectively.

The temperature fluctuated daily, varying from a minimum of 100.8 to a maximum of 106.4 F. The pulse rate ranged from 106 early in the course to 180 in

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the last few days. The consultant on skin diseases suggested that the lesions over the legs resembled a nonspecific type seen in blood dyscrasia. A few days later, small infiltrated, elevated lesions appeared in the skin on the inner aspects of both thighs. One was excised for biopsy (the report follows). Soon after admission, purpuric spots and ecchymotic areas appeared over the entire body. The liver was felt about 2 fingerbreadths below the costal margin. The spleen could just be felt only toward the end of life. The patient gradually lost ground and died on March 29, eighteen days after admission and about eight weeks after the onset of symptoms.

Biopsy.[1]—The serial sections examined revealed a necrotic and inflammatory focus reaching from the corpus papillare into the middle of the cutis. The inflammation gradually decreased at the edges of the sections. The process had nothing characteristic; in type it was mostly productive, consisting of large pale-staining cells (probably adventitial elements), loose pink-staining strands of tissue, clusters of small dark-staining cells and some scattered small areas of necrosis. The inflammation roughly followed the blood vessels. One section had distinct necrosis in the neighborhood of blood vessels, the lesion here being somewhat similar to a tuberculid.

No characteristic tuberculous lesions were found, but acid-fast staining (after autopsy) revealed tubercle bacilli.

Autopsy.[1]—The heart weighed 240 Gm. and measured 10 by 8.5 by 5 cm. The pericardial sac contained about 500 cc. of yellowish fluid in which were flakes of fibrinous material. There were several bright red subepicardial hemorrhages. The heart was small; the subepicardial fat was normal in amount. There was some diffuse thickening associated with a distinct milky spot posteriorly, 3 cm. above the apex of the heart. The ostia and ventricles corresponded in width. The valves were intact. A number of very small hemorrhages were seen in the ventricular septum. They were more distinct directly below the pulmonary valve. In the right auricle, 2.5 cm. above the free edge of the tricuspid valve, there was a round grayish yellow spot, barely visible to the naked eye, surrounded by an indistinct narrow hyperemic zone. No lesions were seen below the pulmonary valve. The coronary arteries were narrow, normal. The ascending portion of the aorta was normal, with little atheroma.

Both lungs were much adherent. The pleura of the left lung was diffusely thickened and grayish. The anterior portions of both lobes did not have coarse thickenings except for a scar in the apex. Posteriorly, however, there were many slightly protruding, partly coalescing round firm nodules with an average diameter of 7 mm. Most of these were situated at the left edge. The apex was occupied by a roughly wedge-shaped firm area, measuring about 4 cm. at the base, with a depth of about 2.5 cm. It was anthracotic, interspersed with small pink soft areas of lung tissue. At the apex proper, there was a cheesy nodule, the size of a hazelnut, with some small cheesy nodules surrounding it. The lung tissue in the anterior halves was well aerated and slightly edematous. Posteriorly, it was mostly the same, with the exception of the apex. There was no evidence of miliary tuberculosis, and the pulmonary veins on both sides had no intimal lesions. The arteries of the lung were normal. The bronchial mucosa was not much injected. The hilar lymph nodes were relatively small and anthracotic. Most of them were soft. In a mediastinal adhesion a firm yellowish gray mass, the size of a large grain of rice, was situated.

1. The biopsy and the autopsy were performed by Dr. Alfred Plaut.

The right lung was larger and firmer. The pleura was diffusely covered by adhesions. The apex was occupied by a large anthracotic and cheesy mass. From the point where this mass came near the hilus, a protruding dark red wedge-shaped area reached down to the lower edge of the lobe. It contained many characteristic fresh peribronchial tuberculous lesions. It was sharply separated from the healthy lung tissue. The lower lobe contained a number of small tubercle-like nodules varying in size up to that of a grain of rice. The hilar nodes reached the size of a small walnut. Some of them contained cheesy foci. No calcification was found.

The organs of the neck were not available.

The spleen weighed 195 Gm. and measured 13.5 by 7 by 3.5 cm. The capsule, which was slightly thickened, showed many somewhat protruding round yellowish and pink nodules, to which on the cut surfaces many firm yellow foci, obviously tuberculous, corresponded. They varied in size up to that of a large pepper seed. There were many which were about twice miliary size.

The adrenal glands were not available.

The left kidney weighed 120 Gm. and measured 10.5 by 5 by 3 cm. The right kidney weighed 120 Gm. and measured 9.5 by 4.5 by 3 cm. The kidneys peeled out of the capsules easily except for one area in each kidney, where the capsule was adherent to some irregular scars. In addition, there was one irregularly outlined yellowish gray focus, the size of a small pea, which possibly was tuberculous, and there were many very small hemorrhages. The main color of the kidneys was a pale pinkish yellow. The cut surfaces were anemic, and the cortical markings were indistinct. The pelves contained some cloudy urine.

The urinary bladder was wide, intact. The urethra was wide and hyperemic. The vagina was normal in width and length. At its lower edge there were several large areas of necrosis. The external os was small, round and nulliparous. The uterus, tubes and ovaries were normal. The peritoneum in the cul-de-sac showed nothing unusual. The rectum was wide. There were some small ulcers directly above the anus, and near the ulcers there was a small fistula, which could be probed for 0.5 cm. only.

The vessels of the stomach, duodenum, bile ducts, pancreas and liver were intact. The bile was a pale olive color and fairly thick. The pancreas was perfectly normal. No tubercles could be seen on the outside or inside. In the surroundings of the pancreas there were several firm, gray and yellow lymph nodes.

The liver weighed 1,620 Gm. and measured 24 by 18 by 7 cm. Through the capsule one saw many small mostly gray tuberculous foci, some of which protruded. The protruding ones were more yellowish. On the cut surface tuberculous nodules were seen as in the spleen but in much smaller number. The acinar structure was coarse, distinct. The vessels of the liver were narrow. The small lobes gave the same picture.

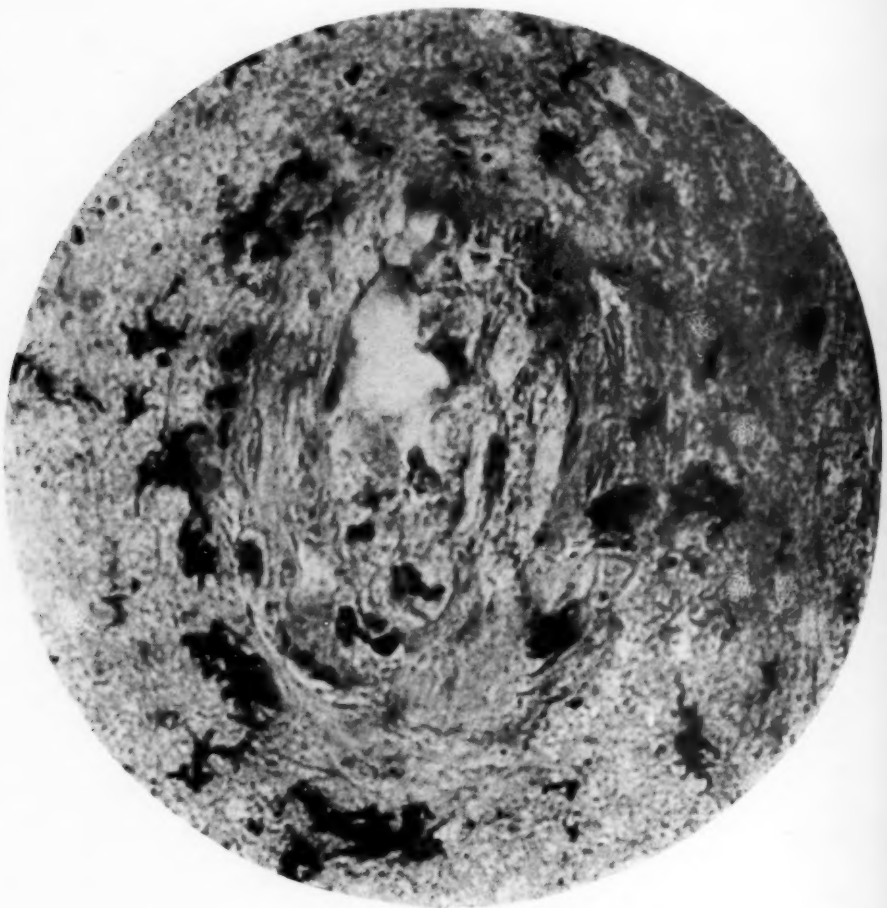
The vena cava was wide, normal.

The large intestine was wide and a few small tuberculous ulcers were seen in it. Ulcerations similar to the ones described in the rectum were situated at the ileocecal valve. Some smaller tuberculous foci, probably ulcerated, were found in the ileum, while the jejunum was intact. In the mesenteric fat, no further tuberculous lumph nodes were seen. The ascending portion of the colon contained several small brownish red polyps. The appendix was intact.

As permission for a complete autopsy was not obtained, no examination of the brain or of the skeleton was possible. (The patient showed evidence of old Pott's disease.)

Microscopic Examination.—The lungs contained a large number of hyaline foci, the average size being from 1 to 2 mm. and the largest 5 mm. In the neighbor-

hood of these foci and occasionally at other points were areas of almost complete necrosis, in which were contained tubercle bacilli, mostly in masses. No tubercles were found. In addition there was a large amount of secondary infection with cocci, as well as severe purulent bronchitis. Several of the necrotic foci were subpleural, but the pleura itself appeared uninvolved. There were moderate edema and hemorrhage. In representative sections from lung tissue which contained small necrotic foci, no primary necrosis of the walls of small arteries was seen.



A necrotic arteriole in the spleen. The black areas correspond to heaps of tubercle bacilli. These masses were visible to the naked eye in the paraffin section (Ziehl-Neelsen stain).

The spleen showed numerous tiny and some larger (up to 2 sq. cm.) areas of necrosis with heaps of tubercle bacilli so large that in stained sections some could be seen with the naked eye. In almost every one of the foci a necrotic artery was seen. Macrophages with red blood corpuscles and blood pigment were found in the foci.

The liver likewise showed scattered areas of necrosis, varying in size, which contained tubercle bacilli in heaps and singly. Surrounding the necrotic parts was a loose stroma. One large focus was situated directly under the capsule. Necrosis, inflammation and heaps of tubercle bacilli extended into the capsule.

In the kidney, corresponding to the yellowish gray pea-sized focus seen in gross, there was a necrotic focus with tubercle bacilli scattered throughout.

In the right auricle of the heart the small nodule just above the free edge of the tricuspid valve had granulation tissue in the outer layers and necrosis in the center. It contained tubercle bacilli. The nodule was situated within the myocardium.

A nodule similar to those described was found in the submucosa of the large intestine. It contained tubercle bacilli.

A small mesenteric lymph node was necrotic throughout and disclosed numerous tubercle bacilli.

The vagina showed a necrotic area more than 1 cm. in diameter, with tubercle bacilli.

Examination of the pancreas, the bones, an ovary and the appendix revealed no necrotic foci. They appeared not remarkable.

It is worth noting that the nodular lesions did not give the usual picture of tuberculous inflammation. They showed almost complete necrosis with thick masses of tubercle bacilli, several of the masses being visible with the naked eye in the stained sections. No tubercles were found.

Diagnosis.—The conditions diagnosed were: Tuberculous sepsis with foci in the spleen, liver, lungs, kidney, myocardium, vagina, intestine and lymph nodes; fibrinous pericarditis; subepicardial hemorrhages; scars in the pulmonary apexes.

COMMENT

Leukemoid Blood Picture in Tuberculosis.—As regards the blood picture with its high percentage of young myeloic cells, one can well understand the temptation to make a clinical diagnosis of acute myelogenous leukemia. Particularly is this true in the present case—that of a severely ill young woman who presented from the outset profound anemia, lesions of the mouth, purpuric spots, high fever, hepatic enlargement and finally splenic enlargement. Accordingly, the diagnosis of acute myelogenous leukemia was entertained until the postmortem examination. However, most of this picture can be attributed to sepsis and in this particular instance to tubercle bacillus sepsis. As for the observations on the blood, many workers have found that tuberculosis may give rise to a myeloid leukemic blood picture with absence of characteristic leukemic infiltrations in the tissues. Marzullo and de Veer [1a] presented two cases of tuberculous infection with a myeloleukemoid blood picture and a clinical course simulating acute myeloid leukemia; in one the infection was pulmonary, in the other, generalized. Other authors have reported instances of a tuberculous infection calling forth young lymphatic rather than myelogenous cells (Wiechman [2]; Landon [3]).

1a. Marzullo, E. R., and de Veer, J. A.: Am. J. M. Sc. **182**:372, 1931.

2. Wiechman, E.: Med. Klin. **18**:1086, 1922.

3. Landon, J. F.: Am. J. M. Sc. **170**:37, 1925.

Necrotic Lesions in Tuberculous Sepsis.—Pathologically the case reported is characterized by widespread caseous necrotic lesions of varying size containing masses of tubercle bacilli. As noted in a foregoing paragraph, no tubercles were found anywhere in the organs. The response to the infecting tubercle bacillus was not a proliferative one. In the literature I have been able to find few reports of cases with similar pathologic observations. I therefore shall go at some length into a discussion of the literature.

Reiche [4] reported the case of a patient with a fever which was continuous for eighteen days, relative bradycardia, constipation, leukopenia, severe anemia and purpura. At autopsy there was a myeloblastic change in the bone marrow. Histologic examination of the organs revealed the interesting fact that the nodules seen in gross (even in the myocardium) in no way showed the typical structure of tubercles but consisted entirely of necrotic tissue, which contained a great number of tubercle bacilli. A case observed by Rennen [5] was similar except that clinically the patient had polycythemia. Lesions were found in the spleen, liver, kidneys and lungs. Scholz [6] described the case of a patient who died after nine days of fever. Microscopic examination of the liver and spleen showed many small necrotic lesions with no structure of a tubercle but with many tubercle bacilli. Balint [7] discussed the case of a patient who exhibited continuous fever for fourteen weeks and then died. At autopsy the pleura, hilar nodes, spleen and liver showed small necrotic lesions with no tubercle structure, the lesions being filled with tubercle bacilli. The rectum exhibited several tuberculous ulcerations. Mönckeberg [8] reported the case of a 23 year old man who had both myeloid leukemia and tuberculosis. Autopsy revealed not only leukemic infiltration in the spleen but also small necroses containing tubercle bacilli.

Friedemann [9] reported the case of a 32 year old man on whom an autopsy was made. The lungs showed many pinhead-sized grayish yellow lesions, with no primary focus or old lesions. The spleen was firm, with many scattered yellowish gray nodules. On the cut surfaces these showed sharply demarcated firm yellowish necroses. The kidneys showed similar lesions. The liver on section presented many irregular, sharply demarcated, necrotic, firm nodules, varying in size up to that of a cherry. At the head of the pancreas were two lymph nodes, each the size of a cherry, which were soft and caseous and in which many tubercle bacilli were demonstrated. In the liver and spleen, microscopically, most of the lesions were necrotic, and several were surrounded by connective tissue. There were no giant cells. The necroses consisted of compact masses with many nuclear rests and contained tubercle bacilli. The other organs showed evidence of miliary tuberculosis, and Friedemann interpreted the findings as primary

4. Reiche, F., cited by Balint.[7]

5. Rennen, K.: Beitr. z. Klin. d. Tuberk. **53**:197, 1922.

6. Scholz, M.: Berl. klin. Wchnschr. **55**:1146, 1918.

7. Balint, R.: Wien. Arch. f. inn. Med. **10**:165, 1925.

8. Mönckeberg, J. G.: Verhandl. d. deutsch. path. Gesellsch. **15**:42, 1912.

9. Friedemann, U.: Arch. f. Verdauungskr. **37**:167, 1926.

reticulo-endothelial tuberculosis (liver and spleen) forming the origin of miliary tuberculosis.

Holzer,[10] in a discussion on typhobacillosis (about which I shall have more to say later), mentioned a case with postmortem observations like those just described. Necrotic lesions with masses of tubercle bacilli were found in the liver and spleen. In addition, there was miliary tuberculosis in the lung, kidney and spleen.

Rich and McCordock [11] referred to one of their cases of very fresh acute caseating miliary tuberculosis in which the lesions were represented by minute cellular accumulations with necrosis. In their case they were able to demonstrate with ease small emboli of clumped bacilli and large numbers of single bacilli in the capillaries of various organs.

Reilly and Bolin [12] reported the case of an infant 10 days old who entered the hospital with an ulcer on the buttock and died after eight weeks' stay. There was a daily swing of the temperature from 101 to 104 F. The white blood count was 19,800 with 78 per cent polymorphonuclears. Tubercle bacilli were found in the stomach contents, and two guinea-pigs that were inoculated with stomach contents and spinal fluid showed generalized tuberculosis at autopsy. At necropsy there was a caseous mass at the apex of the patient's right lung. Throughout the remaining lung there were tiny nodules, from 0.5 to 2 cm. in size. Similar nodules were seen in the liver and spleen. Microscopic examination showed no typical tubercles. The mass at the right apex consisted of caseous material with many round cells, occasional polymorphonuclears and numerous tubercle bacilli. The nodules were not composed of typical tubercles. In the center of each was an area of acute necrosis infiltrated with polymorphonuclear cells. Outside were a few lymphocytes and many polymorphonuclear cells. There were no giant cells but many tubercle bacilli. Similar pictures were found in the liver, spleen and thymus.

Marzullo and de Veer [13] described the case of a 30 year old man who entered the hospital complaining of cough, which he had had for three weeks, fever and weakness. Autopsy showed almost complete absence of productive changes. The tuberculous areas in the lung and spleen were completely necrotic, with little or no epithelioid reaction and no lymphocytic or fibroblastic zones. Tubercle bacilli were present in enormous numbers.

Jakobowicz [13] presented a case of acute tuberculous sepsis in a 54 year old woman. She had high fever for five weeks, but physical examination gave negative results. At autopsy disseminated necroses were found in the lungs, spleen, liver, kidneys, bone marrow of the femur, and mesenteric lymph nodes. There were many tubercle bacilli and rare lymphocytes but no giant cells or epithelioid cells.

The distribution of the lesions in the case which I have reported undoubtedly speaks for tuberculous septicemia. No blood cultures or animal inoculations were made, unfortunately, but nevertheless the

10. Holzer, K.: Beitr. z. Klin. d. Tuberk. **66**:245, 1927.

11. Rich, A. R., and McCordock, H. A.: Bull. Johns Hopkins Hosp. **44**:273, 1929.

12. Reilly, W. A., and Bolin, Z. E.: Am. J. Dis. Child. **41**:582, 1931.

13. Jakobowicz, L.: Beitr. z. Klin. d. Tuberk. **85**:247, 1934.

anatomic picture seems to justify the supposition that the tubercle bacilli were seeded in the numerous organs via the circulation. I was unable, so far as my examination went, to identify a primary focus. However, it is worth noting that three years prior to her death the patient had tuberculous spondylitis, which, however, responded very well to nonoperative treatment, allowing her to be up and at work (dress operator) between her two admissions to the hospital. Courmont [14] mentioned in a discussion of tuberculous septicemia that the origin of the tubercle bacilli producing this condition in man is variable. Sometimes one can accuse an old tuberculous focus. If none is found, it is assumed that sepsis has been present from the start by massive infection.

In discussing the uniformly necrotic nonproductive lesions, Reilly and Bolin [12] offered two explanations:

1. There may be lack of allergic response as gaged by the tuberculin test. The record of the present case shows no such test done. Their patient was an infant.

2. There may be lack of resistance as evidenced by the tremendous number of organisms in each lesion. Adequate resistance, they felt, would not allow the multiplication of the organisms, although these were transplanted by the blood stream. With more resistance, there would be reaction by tubercle formation.

It is not clear, of course, in the latter instance what is cause and what is effect; that is, do the tubercle bacilli on arrival at a focus in tremendous numbers cause caseation around them, or do they flourish and multiply to great numbers in the presence of caseous material about them? It was the opinion of Rich and McCordock [11] that

> a sufficient number of bacilli are essential for necrosis of any appreciable extent. . . . The extent of necrosis in tuberculous lesions will always be a result of a balance between the degree to which allergy is developed and the local concentration of the bacillary products. The number of bacilli necessary to effect necrosis will, in general, be inversely proportional to the degree to which the allergic hypersensitiveness is developed. . . . necrosis may occur entirely independently of allergic inflammation. . . . Necrosis is often merely the direct result of the contact of hypersensitive cells with tuberculoprotein, and needs no preliminary or accompanying inflammation to usher it in.

Further, they added,

> tubercle formation is a type of reaction which is, intrinsically, completely independent of acute inflammation. Tubercle formation is incited by the lipoid extracted from the bacillus, but this lipoid cannot evoke the allergic reaction. For that the tuberculoprotein is necessary.

Rich and McCordock [11] discussed fully the question of the so-called soft tubercle, which I feel may be related to the necrotic type of lesion found in the case which I have reported. It is loosely formed, often poor in cells, with a tendency to early caseation—not the usual tardy caseation of a fair-sized tubercle but necrosis often of even the most minute early accumulation of epithelioid cells. Among fifty cases of early miliary tuberculosis in man, tubercles of the soft variety were shown in seven. The number of bacilli, they felt, is of primary importance in

14. Courmont, P., in Roger, C. H.; Widal, F.; Teissier, P. J., and Garnier, M.: Nouveau traité de médecine, Paris, Masson & Cie, 1921, vol. 4, p. 231.

determining necrosis in tuberculosis. In their animal experiments, even in the face of extreme hypersensitivity, more than a very few bacilli were necessary to effect destruction of tissue to any appreciable degree. Since in a highly allergic body the hard tubercle can develop to a considerable size without necrosis, the extensive and prompt necrosis of the soft tubercle must be due to the presence of a greater number of bacilli within it. Staining their human material, the authors found that bacilli were present in great numbers in soft tubercles, whereas they were extremely scanty in hard ones. They have reproduced soft tubercles in allergic animals by intravenous injection of suspensions of bacilli in which they allowed small clumps to remain in order to cause localization of numbers of bacilli at each site. They concluded that the soft tubercle is merely the result of massive infection of the blood stream in the face of high allergy.

Scholz [6] cited the experiments of Baumgarten on the histogenesis of the tubercle. The latter found that in the rabbit it takes about a week for the tubercle to begin to develop, as indicated by the appearance of epithelioid cells. However, in cells which contained many bacteria mitotic division was not observed, an observation favoring the presence of great numbers of tubercle bacilli as a factor in the lack of tubercle formation.

Jakobowicz,[13] on the other hand, was of the opinion that the number of bacilli is not a factor. He cited autopsy reports in two cases in which tuberculous foci of the pericardium ruptured into the right auricle, in which miliary tubercles were found. In neither of these cases had tuberculous sepsis developed. He favored virulence of the strain of tubercle bacillus as the most important factor and found by animal experiments that tubercle bacilli from persons with acute tuberculous sepsis were more virulent than other strains. Resistance is also a general factor, as most of the cases of acute tuberculous sepsis reported occurred in patients over 45, though some occurred in small children.

In the eleven instances which I have collected from the literature, the youngest patient was 10 days and the oldest 67 years of age. The average age was 39 years. Two others were under 45 years (32 and 30). My own patient was 29 years of age. This varied distribution indicates that the age factor is of no great significance.

FORMS OF TUBERCULOUS SEPSIS

Miliary Tuberculosis.—This has for a long time been accepted by many as an example par excellence of tuberculous sepsis. From some older tuberculous focus, usually giving rise clinically to an appearance of mild tuberculosis, tubercle bacilli are fed to the blood stream. The dissemination results in seeding the tubercle bacilli in the organs, with formation of tubercles. The outcome is usually fatal. As Aschoff [15] stated, one must look for the focus of origin when one is forced to conceive of miliary tuberculosis as a bacillary infection in a more or less immunized body. And here "we know that especially in children we almost always find a so-called intima tubercle in the pulmonary vein or the veins of the adrenal, ductus thoracicus or some other place." This

15. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924, p. 34.

conception is generally upheld. A few pathologists, however, consider the changes in the blood vessels as secondary or as part of the generalized miliary tuberculosis.

Schottmüller defined sepsis as existing only if there is a lesion within the body from which, continuously or periodically, pathogenic bacteria reach the blood stream in such a manner that the invasion of the blood stream results in subjective or objective symptoms. The miliary tubercles form the objective findings which can be considered the results of the septic process. From this standpoint the second stage in Ranke's classification of tuberculous infection is really identical with tuberculous sepsis. The fact alone that bacilli reach the blood stream does not imply sepsis, however, for then most infectious diseases and all tuberculosis would be called sepsis. Since Marmorek showed experimentally that no matter how an animal is inoculated the tubercle bacillus always appears in the animal's blood after a certain time, one can view chronic tuberculosis only as a bacteremia, not as a septic disease.

Typhobacillosis.—Landouzy [16] in 1883 (one year after Koch described the tubercle bacillus) first described as a new form of tuberculous septicemia, distinct from miliary tuberculosis, a typhoid-like condition, common in children, with continued irregular fever, lassitude, chills, drowsiness, an enlarged spleen and frequently an enlarged liver. The abdomen is distended, but the intestinal symptoms are slight. There is leukopenia with relative lymphocytosis. Convalescence may appear to be complete, and yet suddenly or gradually there later appears indisputable evidence of a local tuberculous condition. As Landouzy puts it, the patients live in a condition of latent tuberculosis (*en gestation de tuberculose*), which eventually becomes manifest.

The pathologic lesions as described by Landouzy correspond to findings in septic diseases. There are chiefly hyperemia and degenerative lesions. The lungs and kidneys are hyperemic. The mucosa of the intestine is injected. There is catarrhal inflammation of Peyer's patches. The spleen is soft and enlarged. The liver is large. The heart is flabby and dilated. In the liver and spleen one finds microscopically foci of necrosis which contain tubercle bacilli *en masse*. Sometimes the foci are larger, varying in size from that of a pinpoint to that of a plum. Similar foci of necrosis may be found in the kidney and heart muscle. Occasionally tubercles may be found. It is interesting that, as originally described by Landouzy and subsequently by his school, there were no necrotic lesions in this disease. The German authors, however, have described such lesions persistently as part of the pathologic picture. Landouzy and his school expressed themselves only generally, in that the necrotic nodules are atypical.

Yersin in 1888 reproduced this picture in rabbits by intravenous inoculation of the animals with avian tubercle bacilli. Strauss and Gamaleia and later Pilliet had identical results with guinea-pigs. Gougerot in 1908 reproduced a similar effect in animals with a homogeneous human strain.

As I brought out earlier in this comment on the necrotic lesions, many authors believe that this form of tuberculosis does not show tubercles because the organism has no time to react to the infection with forma-

16. Landouzy, L.: Semaine méd. **11**:225, 1891; Lancet **2**:1440, 1908.

tion of the characteristic tissue. Russew [17] held that this is not true, because in a very rapid course (for example three weeks) there would still be enough time for the structure to be formed. The cause certainly has to be searched for in the causative agent, which according to Löwenstein [18] is the avian tubercle bacillus. In man, the avian tubercle bacillus produces no changes in the organs, at least in the beginning. This bacillus causes a disease which in appearance is nearer to sepsis than to tuberculosis, and in which, after years of septic fever, metastases appear predominantly in the bone marrow, kidney, skin and spleen. Pathologically the changes may be so few that the examiner does not think of tuberculosis but assumes that sepsis is present.

Löwenstein explains why the avian tubercle bacillus has not been identified in these cases by most workers. This bacillus is not pathogenic for the guinea-pig, and the latter animal is the one usually employed for the isolation of the organism. It is pathogenic for rabbits and birds. Furthermore, he has grown it by culture on his special medium.

Balint [7] concluded his excellent paper on tuberculous sepsis as follows:

> Tuberculosepsis shows many forms. The variety is caused partly by the massiveness of the infection and partly by the general and the tissue immunity. The mildest infection runs the course of the juvenile tuberculosis (described by Hollo); the severest infection, the course of general miliary tuberculosis and the fulminating typhobacillosis. Between these two extremes there is a long series of transition forms. The difference between these forms is based clinically on the varying intensity of the general septic symptoms, but anatomically on (1) the more or less abundant appearance of tubercles and (2) on the absence of miliary tubercles. The first-mentioned condition is determined by the intensity of the infection and the degree of the general immunity. The latter is determined by the difference in tissue immunity.

I have gone at some length into a discussion of miliary tuberculosis and typhobacillosis for the express purpose of attempting to fit the picture which I have described into its proper category in tuberculous sepsis. It is not a form of miliary tuberculosis but on pathologic grounds may be linked with fulminating typhobacillosis of the types described by German authors, a condition somewhat removed in their interpretation from typhobacillosis as originally presented by Landouzy. There is left the uncertainty as to the primary source of the infection. Further, I cannot state by what kind of tubercle bacillus my patient was invaded.

SUMMARY

A case is reported of tuberculous sepsis in a 29 year old woman. Necrotic lesions containing numerous tubercle bacilli were found in the lungs, kidneys, spleen, liver, vagina, intestine, mesenteric lymph nodes, myocardium and skin, without any formation of tubercles.

The clinical picture was obscured by a myeloblastic blood picture.

The case is regarded as one of acute tuberculous sepsis not unlike, on pathologic grounds, certain forms of fulminating typhobacillosis described by European authors.

17. Russew, R.: Wien. Arch. f. inn. Med. **20**:231, 1930.
18. Löwenstein, E.: Schweiz. med. Wchnschr. **64**:808, 1934.

Laboratory Methods and Technical Notes

A RAPID AND ECONOMICAL METHOD FOR STAINING ROUTINE TISSUE SECTIONS WITH HEMATOXYLIN AND EOSIN

ARAM A. KRAJIAN, LOS ANGELES

Simplicity and dependability have won for hematoxylin-eosin its place as the standard routine tissue stain. It is dependable because it tolerates wide variations in material and technic. The least fool-proof operations of this time-honored method are the counterstain with eosin and the subsequent dehydration in alcohol. Aqueous or alcoholic eosin stains the tissue comparatively slowly and then is partially extracted by the dehydrating alcohols. The margin of safety between excessive extraction of eosin and complete elimination of the water is not always sufficient to insure a good result.

A change in the manner of applying eosin is described here, which not only corrects this difficulty but also offers the advantages of a more powerful stain with economy of alcohol and of time.

The principle of the modification consists in the dehydration of the section before counterstaining and the application of eosin as a solution in carbolxylene. Then, since the tissue passes afterward through xylene only, there is no opportunity for decolorization. Alcohol is saved because dehydration can be commenced in the stronger alcohol, which obviates the use of the lower gradations. The solution of eosin in carbolxylene, which constitutes the principal element of modification, may be called for convenience eosinol.

PREPARATION OF EOSINOL

Dissolve 5 Gm. of aqueous eosin in 10 cc. of distilled water. Precipitate it by adding 10 cc. of glacial acetic acid and mix with a glass rod. Incubate the resulting coagulum at 56 C. for from twelve to sixteen hours, or until all the water has evaporated.

Dissolve this dehydrated acid-eosin in 10 cc. of absolute alcohol and 20 cc. of acetone, stirring with a glass rod for several minutes. Let the undissolved portion, which is to be discarded, settle to the bottom of the container (about ten minutes). Remove the clear portion with a clean dry pipet; add it to 1,500 cc. of carbolxylene (1 part pure phenol crystals in 3 parts of neutral xylene). Some precipitate will form, which will settle to the bottom of the container. The clear portion is eosinol. The solution keeps indefinitely.

Owing to variation in the staining power of the various brands of powdered eosin, it may be necessary to standardize the solution by staining control sections and adjusting the strength of the eosinol by reducing or increasing the amount of carbolxylene.

From the Los Angeles County Hospital.

STAINING METHOD

1. Prepare microscopic sections by one of the standard sectioning methods (i. e., employing frozen tissue or tissue embedded in paraffin or pyroxylin) and stain in alum-hematoxylin (Harris; Delafield) for from three to seven minutes.

2. Wash in tap water until blue (about thirty seconds).

3. Destain in acid-alcohol (1 cc. of concentrated hydrochloric acid in 99 cc. of 70 per cent alcohol) by dipping the section in and out for even destaining.

4. Rinse in tap water.

5. Blue by dipping in 2 per cent ammonia water (2 cc. of strong ammonium hydrate in 98 cc. of tap water).

6. Wash in tap water for a few seconds.

7. Wipe off the water with a towel, dehydrate in 95 per cent alcohol for one minute and wipe off the back of the slide.

8. Dehydrate completely by pouring on a few drops of absolute alcohol from a drop bottle. Repeat.

9. Counterstain and partially clear in eosinol for from ten to thirty seconds, depending on the strength of the solution.

10. Treat with carbolxylene for three minutes.

11. Treat with first xylene two minutes.

12. Treat with second xylene two minutes.

13. Treat with third xylene two minutes.

14. Mount in gum dammar (saturated solution of gum dammar in neutral histologic xylene [1]).

SUMMARY

By using a solution of eosin in carbolxylene in place of the usual aqueous solution in the hematoxylin-eosin tissue stain, decolorization in the process of dehydration is eliminated at the same time as a more powerful and rapid staining is secured. Alcohol is also saved.

1. The histologic xylene is prepared by the Eastman Kodak Company.

General Review

ICTERUS GRAVIS (ERYTHROBLASTOSIS) NEONATORUM

AN EXAMINATION OF ETIOLOGIC CONSIDERATIONS

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The etiology of icterus gravis neonatorum is still a subject of speculation. The purpose of this study is (1) to evaluate on the basis of a set of criteria formulated from case histories certain of the theories as to the origin of this condition and (2) to examine the evidence as to the fundamental pathologic processes which give rise to the clinical symptoms and to the organic changes found at necropsy. In view of the wide divergence in the opinions of writers on the subject, such an evaluation seems a necessary preliminary to any further investigation concerning the underlying cause or causes of this disease and those related to it. Although adequate accounts of this malady have been given in numerous recent papers (Hawksley and Lightwood [1] and others to be cited), it will be well, before considering the theories of etiology, to summarize the facts regarding the clinical course, the laboratory findings, the pathologic changes found after death, and other common or unique observations which may have a bearing on, or require an explanation in, an acceptable theory of etiology.

CLINICAL OBSERVATIONS

Icterus gravis neonatorum is characterized by marked jaundice, occasionally present at birth, but more often appearing within the first day or two of life. The jaundice usually becomes intense, the color deepening oftentimes to deep bronze. The liver and spleen are usually enlarged, and the spleen may increase further in size during the period of intense jaundice. There may be a low grade fever, or the entire course may be afebrile. There may or may not be petechiae or purpuric spots on the body, and there may occasionally be slight edema, usually in the extremities, eyelids or scrotum. The urine yields urobilin in excess, and often bilirubin and bile salts as well. Albuminuria is exceptional, and oliguria occurs only in rare cases. The stools

From the Women and Children's Hospital.

1. Hawksley, J. C., and Lightwood, R.: Quart. J. Med. **27**:155, 1934.

are not usually acholic, containing varying amounts of urobilin. The icteric index may be elevated to an extreme degree. The indirect van den Bergh test is always positive and the direct reaction variable; a biphasic reaction is most commonly reported. The child at birth may be covered with golden yellow vernix caseosa, and the amniotic fluid may be colored yellow to orange or brown, although this is not invariable. The placenta on gross examination may appear normal or somewhat enlarged.

Another outstanding feature is found in the blood picture. If a blood count is taken soon after birth the number of red cells is usually found lowered to an extent varying from slight to marked as compared with the number found in normal new-born infants, and the hemoglobin, to a relatively less extent. Smears often reveal greatly increased proportions of young and immature red cells—reticulocytes, normoblasts and erythroblasts. Nucleated red cells in excess of 5,000 per cubic millimeter of blood at birth are considered abnormal,[1] and in this condition numbers over 100,000 per cubic millimeter have been reported. Commonly associated with this immaturity of the erythrocytes are anisocytosis, poikilocytosis and polychromatophilia. Leukocytosis is likewise found almost invariably, with a definite shift to the left shown by the hemogram, and often there is an absolute as well as a relative increase in eosinophils and monocytes. The platelets are reduced in number in some cases, while the bleeding time may be normal, slightly prolonged or markedly prolonged, with a normal or increased coagulation time. However, the increased bleeding and coagulation time seem not to be related consistently to the decrease in the platelet count. Fragility tests usually show normal, occasionally slightly increased or decreased, resistance to hypotonic saline concentrations. A normal albumin-globulin ratio was found by Ross and Waugh [2] in a case in which edema was present in the lower extremities.

I. A. Abt [3] stated that "most of these children are strong, robust, and mature at birth." In many cases, however, icterus and enlargement of the liver and spleen may be detected shortly after birth. Cardiac hypertrophy may also be found, and a systolic murmur is sometimes present. The clinical course of the malady in the untreated infant tends most frequently toward a fatal outcome. The stools may become frequent and catarrhal in character, and occasionally vomiting occurs, often projectile in type. As the disease progresses, hemorrhages from the mucous surfaces and into the skin, as well as oozing from the umbilicus, may take place. There may be evidence of gastro-intestinal distress. The cry becomes feeble and whining, respiration may become labored

2. Ross, S. G., and Waugh, T. R.: Am. J. Dis. Child. **51**:1059, 1936.
3. Abt, I. A.: Am. J. Dis. Child. **13**:231, 1917.

and grunting, and spells of cyanosis may develop. The infant appears "drowsy and toxic, difficult to arouse, and refuses or is unable to take the breast or bottle."[4] In contrast to this somnolence and flaccidity, clonic contractures, opisthotonos or even generalized convulsions may occur. Death, following collapse, usually occurs within the first week or two, unless treatment is instituted early and vigorously. Only occasionally has spontaneous recovery been reported (Hawksley and Lightwood;[1] Abt[3]).

The observation of Hawksley and Lightwood[1] should be reiterated at this point. These authors pointed out that in some instances after early treatment with one or more transfusions recovery may seem to be well on the way when more or less suddenly a relapse occurs, indicated by reappearance of symptoms. There may be recurrence of the hemorrhagic tendency, projectile vomiting, evidence of gastro-intestinal distress with frequent stools, cyanosis and labored respirations, and a rise, or in some cases a marked fall, from the normal in temperature, while the cry again becomes high pitched and weak. Such relapses tend to occur most frequently from the third or fourth to the sixth week and have been reported as late as the tenth week. More than one such recurrence may take place. Prompt amelioration usually follows one or more additional transfusions.

Although some of these infants may be premature, the majority are born at term, with usually a normal and frequently a short and easy delivery. There is no evidence of asphyxia at birth. The parents are healthy. The Wassermann and Kahn tests of the parents as well as of the baby are negative. Tuberculin tests of the infant are also negative. The mother usually has had an uneventful pregnancy, and age, dietary deficiencies and anemia in the mother cannot be ascribed as causative factors; nor can infection of the infant in its passage through the birth canal. Wanstrom,[5] for example, reported the condition in the infant of a 17 year old girl, and A. F. Abt[4] cited Smyth's case of an Australian mother who had given birth to 9 children; the first 2 were normal, the third was premature and stillborn, while in the next 6 icterus gravis neonatorum developed and all died in from three to eleven days after birth. During the entire course of her tenth pregnancy the mother was hospitalized under extremely careful supervision and with regulation of her diet, and she had an entirely normal pregnancy. Seven days before labor was due a cesarean section was performed and an apparently normal child delivered. The infant remained normal for twenty-four hours; then it became drowsy and increasingly icteric, and died seventy hours after birth.

4. Abt, A. F.: J. Pediat. **3**:7, 1933.
5. Wanstrom, R. C.: Am. J. Path. **9**:623, 1933.

There is no direct evidence that a hereditary factor determines this condition. Hilgenberg's case, cited by A. F. Abt,[4] of a mother who had had several normal children by a first marriage and who following a second marriage gave birth to a number of babies that died of icterus gravis neonatorum does not prove that the second father was biologically implicated, since the same sequence might have occurred with the first husband as father. Gierke's case, also cited by Abt, in which a man with a normal child by a first wife had, in a second marriage, children in whom icterus gravis neonatorum developed does not allow the presumption of a hereditary taint in the second wife. Hoffmann and Hausmann [6] stated that the parents in one of their affected families (family B) were related, but the possibility of familial acholuric jaundice was not ruled out in this case, in which the erythrocytes showed decidedly increased fragility. Hampson [7] noted that this hereditary malady, while it does not usually affect severely the new-born infant, may cause death during the first few weeks of life from progressively increasing jaundice. This occurred in all the children in three families known to him, the mother in each case showing diminished resistance of the red cells to hypotonic saline solutions. Hawksley and Lightwood [1] included in their series a case in which the parents were cousins and there was a history of fatal neonatal jaundice in the preceding generation (case 18), but it is questionable whether the infant in this case had the grave type of jaundice, since jaundice did not appear until the third day, there was no enlargement of the liver or spleen, there were no nucleated red cells in the blood smear, no leukocytosis was present and the infant recovered uneventfully. Hoffmann and Hausmann,[6] however, reported another case (family X) in which the affected infant was the grandchild of a woman whose sister lost 2 babies with severe jaundice on the fourth day of life. Examples reported in early series of cases, on the other hand, before an accurate diagnosis of syphilis was possible, must be considered as questionable. Further evidence purporting to relate this disease to certain genes or, less specifically, to a defect in the germ plasm, will be considered later.

While in most cases of this disease there is nothing in the family history suggestive of a hereditary influence, there is, on the other hand, a definite familial incidence. The term "familial" is used in this connection to designate the tendency for the disease to recur in children of subsequent pregnancies of a mother who has already given birth to a child either with this malady or with one of the apparently related conditons, congenital edema or congenital anemia of the new-born. Clifford and Hertig [8] stated that the first child is usually spared and

6. Hoffmann, W., and Hausmann, M.: Monatschr. f. Kinderh. **33**:193, 1926.
7. Hampson, A. C.: Lancet **1**:429, 1929.
8. Clifford, S. H., and Hertig, A.: New England J. Med. **207**:105, 1932.

that this occurs so frequently as to seem significant. A number of authors, however, have reported cases of the condition in first-born infants, among them May, cited by I. A. Abt,[3] Buhrman and Sanford[9] and Andrews and Miller.[10] In addition to the familiar instances of this malady, sporadic cases seem also to occur, but the ratio of the incidence of the sporadic to that of the familial type has apparently not been determined, nor has it been demonstrated that the etiology of the sporadic is or must be identical with that of the familial form of the disease.

That this disease is not extremely uncommon is shown by the statistics of Clifford and Hertig,[8] who in 1932 reported 7 proved cases of icterus gravis neonatorum associated with erythroblastosis in 2,400 new-born infants in nine months, an incidence of 1 in 340. Andrews and Miller[10] reported 4 cases in 1,958 new-born infants, a rate of 1 in 490. These figures do not include congenital edema, which is of less frequent occurrence, reported as observed once in 2,000 and once in 3,000 new-born infants by Stoeckel and Döderlein[11] and once in 1,200 new-born infants by Clifford and Hertig.[8] The disease has been reported in many countries. Ku and Li[12] reported it in a child of Chinese parentage, and Andrews and Miller[10] found it in 2 Negro infants.

PATHOLOGIC OBSERVATIONS

Necropsy of infants with this condition reveals, aside from the deep yellow-green staining of all the internal viscera, mucous and serous membranes, skin, scleras and fat tissues, little gross change other than enlargement of the liver and spleen. Pallor, however, is usually noted to a varying degree in spite of the jaundice, and petechial and small ecchymotic hemorrhages are frequently found in the skin and in the mucous and serous membranes.

The brain is usually pale, but in a certain relatively small number of cases, according to Schmorl,[13] the brain substance takes on a more or less intense coloration, appearing as if inbibition of the yellow pigment had taken place. On microscopic examination the whole tissue appears colored uniform pale yellow, while bilirubin crystals may be found in the blood vessels and in the spinal fluid. It cannot be demonstrated that this color is bound to any special structural elements. In this diffuse icterus of the brain there are foci of fat granules, appearing as opaque yellow flecks, distributed symmetrically in the medullary

9. Buhrman, W. L., and Sanford, H. N.: Am. J. Dis. Child. **41**:225, 1931.
10. Andrews, H. S., and Miller, A. J.: Am. J. Dis. Child. **50**:673, 1935.
11. Stoeckel and Döderlein, cited by Clifford and Hertig.[8]
12. Ku, D. Y., and Li, Y.: Virchows Arch. f. path. Anat. **283**:62, 1932.
13. Schmorl, G.: Verhandl. d. deutsch. path. Gesellsch. **6**:109, 1904.

layers of the hemispheres, lateralward from the central ganglions. They are localized, occurring outside of this region only exceptionally, and are more numerous in the more posterior sections. In microscopic sections these foci appear as areas of softening. In addition to the fat granules there are occasional red blood cells and more or less numerous intracellular and extracellular pigment granules and bilirubin crystals. Occasionally a nest of fat granules shows peripherally an infiltration of round cells. These foci stand in close relation to the blood vessels. Frequently, in the region of a focus, the walls of the blood vessels reveal hyaline degeneration, with thrombi in the lumens, which appear partly as homogeneous shining masses containing leukocytes in the process of destruction and partly as fibrin in the meshes of which white blood cells and platelets are held. It is not known whether the thrombi are constant nor whether they stand in a pathologic relation to the foci. These foci of fat granules, however, are associated with icterus, since they occur only simultaneously. Although some authors attributed them to sepsis of the spinal cord on an embolic basis, Schmorl was not inclined to do so, since he had not especially observed sepsis of the cord in icteric children on whom he had performed necropsies, and he could demonstrate bacteria in the regions of the foci neither through culture nor through staining.

In addition to this diffuse icterus of the brain there is a second type in the new-born in which the yellow coloring is circumscribed, being limited entirely to those areas of the brain where larger groups of ganglion cells are found, that is, to the nuclear areas. This type Schmorl designated as *Kernikterus*. The cortex of the cerebrum and cerebellum, the head of the striate body, the caudate nucleus and the optic thalamus are free from pigment, according to Schmorl. Zimmerman and Yannet,[14] on the other hand, found that the structures most commonly affected are the caudate, lenticulate, subthalamic and dentate nuclei, the thalami, the mamillary bodies, the cornua ammonis, the nuclei of the cranial nerves, the olives and even parts of the cerebellar cortex, as well as the anterior and posterior horns of the spinal cord. Many of the colored ganglion cells show necrotic changes, and the axis cylinders are especially intensely colored. Schmorl felt that the sharp demarcation of the pigmentation shows that this yellow coloration is not related to simple imbibition of bile pigment but is bound to definite structural elements. He found, furthermore, that when this nuclear icterus was noted the yellow pigment in the ganglion cells did not turn to the green of oxidized bilirubin in formaldehyde and mercuric chloride solutions. This was likewise true in regard to all the organs, including the liver, which became yellow-brown, although in the fresh

14. Zimmerman, H. M., and Yannet, H.: Am. J. Dis. Child. **45**:740, 1933.

specimens they had not differed in appearance from the organs of other icteric infants who did not show this nuclear staining in the brain. Furthermore, the pigment in these nuclear elements reacted only faintly or not at all to nitric acid (Gmelin test). Schmorl felt, therefore, that this yellow color was not due to the ordinary bilirubin but possibly to a modified bile pigment. Schmorl found this condition in 6 of 120 necropsies in cases of jaundice of the new-born.

Much more frequent than nuclear icterus, however, may be spontaneous intracranial hemorrhage, which was found in 6 of 18 cases reported by Hawksley and Lightwood.[1]

The liver, which may be enlarged to two or three times the normal size, presents very often a pathologic picture which has given rise to considerable speculation regarding the underlying cause of this disease. Frequently outstanding in this organ on microscopic examination are large numbers of what appear to be hematopoietic masses, usually predominantly erythropoietic, scattered in the periportal spaces and within the hepatic sinusoids. These islands of blood cell formation may be so numerous as to compress and narrow the cords of hepatic cells, sometimes distorting greatly the general architecture of the liver. This marked extramedullary hematopoiesis is most commonly found in the apparently related condition designated as universal edema of the fetus (fetal hydrops). In icterus gravis neonatorum, on the other hand, it is sometimes only moderate and occasionally almost entirely absent. This fact is remarked by Hawksley and Lightwood.[1] It is demonstrated in the first case of Zimmerman and Yannet,[14] in which were found "but few islands of blood formation," and even more clearly in a case recorded by de Lange,[15] in which the liver of a twin who died revealed "an entirely isolated small focus of hematopoiesis in one capillary loop . . . and a single red blood cell showing amitotic nuclear division in a sinusoid, certainly no more than in normal new-born infants of this age." In a few cases the extramedullary islands of blood formation have seemed to consist mostly of cells of the leukocytic rather than of the erythrocytic series, as in the case of "leukoblastosis" described by Gierke;[16] in others neutrophilic leukocytes share more or less equally with the red cells in the extramedullary hematopoiesis, as in the cases of "erythroleukoblastosis" described by Salomonsen,[17] Wanstrom[5] and others.

The liver cells themselves may appear unchanged,[18] or they may show damage varying from cloudy swelling and fatty degeneration to

15. de Lange, C.: Jahrb. f. Kinderh. **145**:273, 1935.
16. von Gierke, E.: Virchows Arch. f. path. Anat. **275**:330, 1930.
17. Salomonsen, L.: Ztschr. f. Kinderh. **51**:181, 1931.
18. de Lange, C., and Arntzenius, A. K. W.: Jahrb. f. Kinderh. **124**:1, 1929.

outright diffuse necrosis and disintegration as seen in Klemperer's first case.[19] The biliary canaliculi may be distended with bile, presenting the so-called bile thrombi, although these are not invariably found; Diamond, Blackfan and Baty, for example, found them absent in 2 of 4 cases at necropsy.[20] The central veins and the blood capillaries may be dilated, as observed in several instances by de Lange and Arntzenius [18] and in MacClure's case.[21] Many of the liver cells contain bile pigment as well as numerous granules which on proper staining prove to be hemosiderin. A case was reported by de Lange and Arntzenius in which occasional granules of iron pigment lay free in the blood spaces. The Kupffer cells may be increased in number and are often distended with bile and iron pigment. They may become loosened and lie free in the sinusoids. Frequently they exhibit erythrophagocytosis, and de Lange and Arntzenius [18] and MacClure [21] found erythrorrhexis and erythrocytolysis. Eosinophils may be numerous in the periportal spaces and are sometimes found associated with the islands of blood formation. In 7 of 9 cases in which the period of survival was five weeks or longer Hawksley and Lightwood [1] observed a fine fibrosis among the polygonal cells of the atrophic areas and near the portal tracts.

The spleen is greatly enlarged and engorged with blood. The malpighian bodies may be hardly visible. Microscopically, the germinal centers appear to be decreased in size and number and more widely separated than in the normal spleen. Many red cells are engulfed in phagocytes, and numerous macrophages contain bile and in some cases iron pigment also. Here, likewise, masses of hematopoietic cells may be found.

The heart is frequently larger than normal. There are usually no septal defects. Petechial hemorrhages are often visible on the epicardium. In an occasional case edema of the muscle bundles has been observed.[20]

The lungs in many of the reported cases have shown hemorrhages varying from subpleural petechiae and small dark brown ecchymotic areas to fairly extensive hemorrhages involving a portion of a lobe. Areas of "bronchopneumonia" have also been reported, while in one case Diamond, Blackfan and Baty [20] found edema of the interstitial tissue, and in another, nucleated red cells in groups within the alveolar walls.

The kidneys, pancreas, thymus, thyroid, adrenals and lymph glands have also been reported as having foci of hematopoiesis. Ross and

19. Klemperer, P.: Am. J. Dis. Child. **28**:212, 1924.
20. Diamond, L. K.; Blackfan, K. D., and Baty, J. M.: J. Pediat. **1**:269, 1932.
21. MacClure: Ztschr. f. Kinderh. **51**:86, 1931.

Waugh [2] described a case in which myeloid elements were present in the adventitia of large blood channels in the thymus, lungs, kidney and adrenals and in the tissue surrounding the hypogastric vessels. The kidney may reveal siderosis, and bile casts may be found in the tubules. Parenchymatous degeneration in this organ is infrequently but occasionally reported.

The bone marrow reveals active hematopoiesis, often in excess of normal, in some cases causing overcrowding of the marrow spaces. Buhrman and Sanford [9] reported numerous megakaryocytes in a case, although occasionally a diminution in their number has been observed.

This picture of unusual and occasionally extreme extramedullary blood cell formation is regarded as abnormal for a full term infant. The condition is that usually found in a 5 month fetus. Although slight to severe anemia exists in these children, there is an increased output of young and often quite immature red cells, not only from the bone marrow but presumably also from the numerous erythropoietic foci in many visceral organs. This is the origin of the descriptive term "erythroblastosis." It is associated with enlargement of the liver and spleen, pallor of the tissues and in some cases jaundice or edema or both, although Ferguson,[22] Salomonsen [17] and de Lange [23] have each reported cases in which marked erythroblastosis was found on necropsy with neither hydrops nor jaundice.

FAMILIAL ASSOCIATIONS

On the basis of these facts one may now consider a possible relationship between icterus gravis neonatorum and congenital edema of the new-born (fetal hydrops), on the one hand, as pointed out by Gierke [16] and Eichelbaum and concurred in by Salomonsen,[17] de Lange [23] and others, and icterus gravis and the so-called idiopathic anemia of the new-born, on the other hand, as noted by Grulee and by Diamond, Blackfan and Baty.[20] Pathologic examination reveals a much more fulminating process at work in congenital edema than in icterus gravis neonatorum. There are, however, definite points of similarity. Edema, which is marked in hydrops, is sometimes seen in icterus gravis, although when present it is usually slight and localized. Jaundice is more pronounced in icterus gravis but often present in hydrops. The liver and spleen are enlarged in both conditions. The placenta is usually greatly enlarged in universal edema and may be slightly enlarged in grave jaundice. Anemia is found in both disorders, and extramedullary hematopoiesis and circulating immature red blood cells may be a feature in hydrops as in icterus gravis. Both conditions like-

22. Ferguson, J. A.: Am. J. Path. **7**:277, 1931.

23. de Lange, C.: Acta pædiat. **13**:292, 1932.

wise reveal a familial tendency and have been reported by a number of authors as occurring in siblings. Salomonsen,[17] among others, reported the case of a family in which healthy parents had 4 children, the first of whom was living and well, while the second had icterus gravis, the third hydrops with erythroblastosis and the fourth icterus gravis. De Lange [23] reported the case of twins, one of whom was born with fetal hydrops, while the other presented icterus gravis soon after birth. In a recent study by Macklin,[24] both conditions were found in 14 families reported on in the literature. Such a familial association of two conditions in which the pathologic changes differ in degree rather than in kind is difficult to explain except on the basis of an identical etiology.

The evidence in regard to a close association between icterus gravis and congenital (idiopathic) anemia of the new-born is also suggestive. The latter condition, as defined by A. F. Abt,[25] is "a severe anemia manifesting itself usually within the first two weeks of life in an infant in whom may be excluded the accepted causes of secondary anemia, namely sepsis, syphilis and hemorrhagic disorders." Erythroblasts may be present in the circulating blood. Mild icterus, enlargement of the liver and spleen, and extramedullary hematopoiesis are frequent. Stransky [26] expressed the belief that there are two types of primary anemia of the new-born. The first and more frequent type is characterized by a nonregenerative blood picture, with no true shift to the left and no embryonal cells, which he ascribed to a constitutional insufficiency of the bone marrow. The second type, which is more rare, shows an embryonal blood picture, with areas of hematopoiesis in the liver, spleen and lymph glands. That these two types represent differing individual responses to a similar etiologic factor is indicated by the appearance of both types in children of the same parents. Abbott and Abbott,[27] for example, reported a case in which the family history revealed such a relationship:

> The first child in this family exhibited a yellowish tinge of the skin at birth. Slight fever developed on the second day, and cough and bleeding from the nose on the fifth day, when he died. Necropsy revealed in the lungs "a hemorrhagic bronchopneumonia" and in the liver a number of fetal blood islands in the lobules and nucleated red cells in the capillaries. The spleen, kidneys and peribronchial lymph nodes were normal, and bacteriologic examination of the spleen and bone marrow gave negative results. The second child gradually became pale and took food less well, dying on the eighth day. On necropsy numerous blood islands were found in the liver, but they were of myeloblastic and myelocytic elements, with nucleated red cells conspicuously few. A heavy infiltration of eosinophils and myelocytes was found in the portal spaces. The spleen was enlarged, with the

24. Macklin, M. T.: Am. J. Dis. Child. **53**:1245, 1937.
25. Abt, A. F.: Am. J. Dis. Child. **43**:337, 1932.
26. Stransky, E.: Ztschr. f. Kinderh. **51**:239, 1931.
27. Abbott, K. H., and Abbott, F. F.: Am. J. Dis. Child. **49**:724, 1935.

malpighian corpuscles inconspicuous. All other organs were essentially without change. The third pregnancy ended in a spontaneous abortion. The fourth child was the one reported. The mother's pregnancy had been supervised with great care and was entirely uneventful. The child at birth appeared well nourished, cried lustily and was of a normal, ruddy appearance. A blood count on the first day revealed only an occasional normoblast, with red cells 4,136,000, leukocytes 13,600 and hemoglobin (Sahli) 82. A differential count according to percentage showed polymorphonuclear neutrophils 55 (segmented forms 42, stab cells 11, juvenile forms 2), lymphocytes 37, monocytes 6, eosinophils and basophils 0. On the fifteenth day pallor was noted. There was no jaundice, no hemorrhagic manifestation, no bile in the urine, and the stools were normal. The liver was slightly enlarged, the spleen palpable and the inguinal and axillary lymph nodes slightly enlarged, but no demonstrable focus of infection could be found. Hemograms showed "marked primary anemia," which grew progressively worse in spite of the administration of blood intramuscularly and of liver extract. On the twenty-third day the red cell count was 1,664,000, the leukocyte count 10,200 and the hemoglobin (Sahli) 36. There were no normoblasts, no reticulocytes and no myelocytes. On the twenty-ninth day the anemia was less marked, there was 1 normoblast per hundred white cells, and the reticulocytes were designated 3+. From this time on recovery was progressive until complete.

Here one has a definitely nonregenerative congenital anemia associated familially, on the one hand, with a congenital anemia exhibiting erythroblastosis and, on the other, with one presenting the pathologic picture designated as "leucoblastosis" by Gierke. Diamond, Blackfan and Baty [20] likewise presented an interesting family series, the first 4 children being normal, the next 3 showing congenital anemia of the regenerative type, and the eighth exhibiting all the clinical symptoms of icterus gravis neonatorum. These authors have called attention to the fact that congenital anemia presents a clinical picture identical with that found in an infant with icterus gravis in whom treatment has brought about disappearance of the jaundice. Finally, Pasachoff and Wilson [28] presented a report showing the extraordinary combination of congenital anemia of the new-born and fetal hydrops in offspring of the same parents, and it is noteworthy that erythroblastosis was not present in either child.

The association between edema, icterus gravis and anemia of the new-born was postulated by Diamond, Blackfan and Baty [20] on the basis of what they considered the common underlying pathologic condition, erythroblastosis. There have, however, been cases reported of each of the three disorders in which there has been little evidence of abnormal erythroblastic activity.[29] Furthermore, as Pasachoff and Wilson [28] pointed out, erythroblastosis is by no means pathognomonic of this group of disorders, for it occurs in other conditions as well, notably

28. Pasachoff, H. D., and Wilson, L.: Am. J. Dis. Child. **49**:411, 1935.

29. Zimmerman and Yannet.[14] de Lange.[15] Abbott and Abbott.[27] Pasachoff and Wilson.[28]

in congenital syphilis and in sepsis of the new-born. It seems rather that it is the familial association of these three conditions which constitutes the most convincing evidence for an etiologic relationship, and this association must be taken into consideration in the evaluation of theories as to etiology.

THEORIES AS TO ETIOLOGY

The foregoing consideration of the symptoms, clinical course and pathologic changes found in icterus gravis neonatorum, together with its familial association with the apparently related diseases, fetal hydrops and idiopathic anemia of the new-born, may now constitute a foundation for a critical evaluation of some of the theories as to its etiology. It may be stated that any attempted explanation of its cause must allow for the following observations: (1) the apparent absence of any hereditary factor; (2) the fact that birth of healthy, normal children may precede that of the child in whom the condition first manifests itself in a family; (3) the frequent familial tendency; (4) the apparent health of the parents; (5) the apparent absence of significant factors in the prenatal history in the large majority of cases; (6) the presumptive association of edema, grave jaundice and anemia of the new-born; (7) the clinical symptoms; (8) the observations at necropsy, and (9) the erythroblastosis.

The theories as to etiology which have been proposed in the literature fall into two groups, (1) those implicating the mother primarily and (2) those finding the cause solely within the child.

Theories Involving the Mother.—Under this heading one finds ascribed as possible causative factors:

1. Nutritional disturbances in the mother, brought about either by dietary deficiencies or by frequent gestations and lactations. Parsons,[30] for example, holds that maternal anemia resulting from a long-deficient diet is the most probable cause of congenital anemia in the offspring, and other authors have recommended the administration of liver or iron or both to the mother before birth of the child and to the infant after birth. In the large majority of cases, however, the mother presents a normal blood picture, and since a close supervision of the diet has been exercised in numerous cases,[31] one can consider neither anemia nor nutritional disturbances in the mother as primarily operative in the causation of icterus gravis neonatorum or of the presumably related conditions.

2. Disease in the mother. Some of the earlier writers, attempting to assign a cause for icterus gravis neonatorum, failed to differentiate

30. Parsons, L. G.: Acta pædiat. **13**:378, 1932.

31. Smyth's case, cited by Abt.[4] Abbott and Abbott.[27]

this disease from a similar condition found in syphilitic infants and ascribed it to syphilis. Syphilis, however, has been repeatedly ruled out, not only by the negative serologic tests of the parents' and of the child's blood, but by the entire absence of spirochetes in sections of organs stained by the Levaditi method. Tuberculin tests of the infant have likewise been consistently negative, and there is no pathologic evidence of tuberculosis on necropsy. Acute infection in the mother, on the other hand, might be a factor in the sporadic case, but it does not account for the frequent familial tendency.

3. Toxemias of pregnancy. Toxemia has been manifested in a certain proportion of cases and has been considered as the causal factor by Rolleston,[32] Hoffmann and Hausmann[6] and others. Among the symptoms reported in the mother during the pregnancy from which an affected child was born may be listed edema, usually of the lower extremities, as noted by de Lange, jaundice, reported by Rolleston in 15 of 130 collected cases, nephritis, observed in 2 cases by Gierke,[16] severe pain, reported by MacClure,[21] and frequent attacks of faintness together with marked weakness, as in Zimmerman and Yannet's[14] second case. In the case reported by Bullard and Plaut[33] the mother, after giving birth to 1 normal child and 2 in whom icterus gravis neonatorum developed, all 3 pregnancies being uneventful, suffered during her fourth pregnancy three attacks diagnosed as "Ménière's disease, classical and unmistakable" and was troubled with edema of the feet and with constant nausea and insomnia, but with no ocular symptoms and no headaches. The blood pressure, urine and blood picture were normal. She finally delivered prematurely a stillborn hydropic fetus. The mother of 4 children whose cases were reported by Diamond, Blackfan and Baty[20] felt marked lessening of fetal movements before the birth of each of these children, although this had not occurred with the first 4 of her offspring, who were unaffected. In 2 cases known to me abnormal spotting occurred at 4 and 4½ months, respectively. It seems that such incidents may have an explanation other than as mere accidents. On the other hand, by far the largest number of reports describe the mother's pregnancy in these cases as normal and uneventful. Hampson,[7] accordingly, suggested that the mother's symptoms may be secondary, resulting from carrying an anomalous fetus.

Theories That Locate the Cause in the Child.—Woolley[34] and Oberndorfer[35] each reported a case of fetal hydrops involving twins. Since it has been shown that this condition appears to be related etio-

32. Rolleston, H.: Brit. M. J. **1**:864, 1910; Practitioner **104**:1, 1920.
33. Bullard, E. A., and Plaut, A.: Arch. Pediat. **43**:292, 1926.
34. Woolley, P. G.: J. Lab. & Clin. Med. **1**:347, 1916.
35. Oberndorfer, S.: Zentralbl. f. Gynäk. **51**:1830, 1927.

logically to icterus gravis neonatorum, their observations are of interest. In each instance an edematous twin, manifesting fetal hydrops with erythroblastosis, was accompanied by an apparently normal twin. Oberndorfer felt that such an occurrence speaks against maternal influence in the causation of erythroblastosis and that the cause must in truth be sought in the fetus. His argument appears to be strengthened by the fact that in a still later pregnancy the edema which the mother of the twins whose case he reported invariably experienced from the fourth month in each pregnancy in which the fetus was affected disappeared in the fifth month with the cessation of fetal movements, and was followed two months later by the delivery of a macerated fetus, apparently long dead. It must be considered, however, that in neither Woolley's nor Oberndorfer's case was the apparently normal twin examined at necropsy, although in Woolley's case the infant was stillborn, and presumably Oberndorfer's "normal" twin, which was premature (7 months), likewise did not survive. Since de Lange [23] reported a case in which one twin was born with fetal hydrops and the other presented icterus gravis after birth, it is entirely possible that the supposedly normal twins in the cases reported by Woolley and Oberndorfer would, had they lived, have manifested the latter condition. Macklin [24] found in the literature 7 cases of twins in 5 of which both twins were affected with icterus gravis; in the sixth case one twin was icteric and the other hydropic, and in the seventh case icterus gravis developed in one twin, while the other died at birth. With respect to congenital edema, however, she found one twin affected and the other normal in 8 of 9 cases. Whether the unaffected twin in each case was born living and remained alive and normal is not stated, nor were the sources of these statistics tabulated.

The search for the cause of icterus gravis may now tentatively be shifted from the mother to the offspring, and indeed there is a predominance of theories based on some presumed anomaly in the child:

1. Some of the older theories of jaundice in the new-born were based on the conception set forth by Eppinger that the bile pigment was elaborated by the liver cells and that the jaundice was the result of obstruction either in the extrahepatic ducts or within the liver, where blocking of the canaliculi was thought to be caused by the formation of bile thrombi by inspissated bile. It is now known that the pigment bilirubin is formed outside of the hepatic epithelial cells, probably by the reticulo-endothelial cells, especially those in the liver, spleen and bone marrow, from hemoglobin, and that jaundice may result from an abnormally rapid breakdown. When this occurs in the absence of obstruction or of actual necrosis within the liver the direct van den Bergh reaction of the serum is negative while the indirect

reaction is positive. Rich [36] stated that "no form of jaundice is known which results from a purely functional depression of the excretory power of the liver, that is, without actual loss of cells through necrosis, when the production of bilirubin is in normal amounts." Hence one must infer that when only the indirect or, delayed, reaction is found strongly positive one is dealing with excessive production of bilirubin or, in other words, with increased destruction of erythrocytes. This explanation is accepted generally for icterus simplex neonatorum. Since the positive indirect van den Bergh reaction is likewise constant (to a degree greater than normal) in the malady under discussion, while the direct reaction has in some instances been reported negative, as in Buhrman and Sanford's [9] cases, one can for the same reason no longer ascribe the jaundice in icterus gravis neonatorum to obstruction only, but must here also recognize increased destruction of red cells as an important factor. Such a conclusion, moreover, is substantiated by the observation of increased erythrophagocytosis, erythrorrhexis and even erythrolysis [37] in the liver and in the spleen, as well as of hemosiderosis in these organs, and by the significant observation of A. F. Abt [4] in one of his cases that the amount of hemoglobin as calculated from the blood iron was greater than the amount of hemoglobin actually found by the Newcomer method, a finding confirmed as abnormal by Sobel.[38] Pfannenstiel [39] held that icterus gravis neonatorum is simply an intensification of the physiologic jaundice of the new-born, but it seems that the jaundice in icterus gravis neonatorum is the result of a pathologic process of a different character from that resulting in icterus simplex, as indicated not only by the higher icteric index and the frequently positive direct van den Bergh reaction but also by the production of anemia, in some cases extremely severe, even in the presence of greatly increased hematopoiesis. Furthermore, in icterus gravis neonatorum there is frequently evidence of injury to various organs, especially to the liver. That this injury to the liver is not secondary to obstruction alone is suggested by the much less rapidly devastating, although ultimately fatal, course of the jaundice due to congenital atresia of the bile ducts, as pointed out by Yllpö,[40] an observation confirmed recently in a case reported by Kass and Osgood,[41] in which an infant lived to the age of 11 months and 29 days with complete obliteration of the cystic, hepatic and common ducts and a nonfunctioning gallbladder.

36. Rich, A. R.: Bull. Johns Hopkins Hosp. **47**:338, 1930.
37. de Lange and Arntzenius.[18] MacClure.[21]
38. Sobel, I. P.: Am. J. Dis. Child. **51**:104, 1936.
39. Pfannenstiel, J.: München. med. Wchnschr. **55**:2169 and 2233, 1908.
40. Yllpö, A.: Ztschr. f. Kinderh. **17**:334, 1918.
41. Kass, I. H., and Osgood, F. P.: J. Pediat. **9**:91, 1936.

2. Gierke [16] considered as the cause of both fetal hydrops and icterus gravis neonatorum a "primary constitutional (familial or hereditary) anlage defect of the hematapoietic system." Salomonsen [17] was inclined to agree with him, and in this country Clifford and Hertig [8] felt that such a defect is the most likely cause, stating that "an unknown stimulus manifests itself in late uterine life as a pathologic prolongation of, or reversal to, the embryonic system of blood formation. As a result abnormally young forms of red cells appear in the circulating blood. Concomitant with this abnormal production there is an increased destruction of the red cells." A. F. Abt,[4] likewise, bases his theory of "embryonal hematopoietic persistence" in the familial type of case on a defective anlage or a defect in the germ plasm.

It is reasonable to assume a defect in the germ plasm in these related diseases since such a defect provides an explanation for their appearance in subsequent children of the same family. It is therefore necessary to examine this hypothesis in some detail. In considering this explanation it is important to remember, as Clifford and Hertig pointed out, that the first child is usually spared. This is frequently true of the second as well, and Diamond, Blackfan and Baty,[20] indeed, recorded a case in which 4 unaffected children preceded the first child with congenital anemia, who was followed by two more with the same condition and an eighth child, in whom icterus gravis neonatorum developed. There is no history of infection, exposure to roentgen rays, evidence of endocrine dysfunction or other baleful influence on the germ plasm in the mother to account for the appearance of the malady in later children. Hawksley and Lightwood [1] suggested the possibility of a mendelian recessive characteristic as responsible, but the incidence of the condition in a large family or in a series of families is too large, as Macklin [24] pointed out. This author recently undertook a statistical study of these diseases and came to the conclusion that they are inherited rather as a dominant mutation. By including with the hydropic infants all miscarriages, stillborn infants and infants showing erythroblastosis in the families furnishing the data on fetal hydrops and eliminating from her group with icterus gravis neonatorum all miscarriages, stillborn infants, infants with hydrops and icteric babies who recovered, she found the incidence of each of these maladies in a large group of families to be approximately 50 per cent, the incidence required theoretically, she stated, if these diseases are due to a dominant mutation. She suggested further that the diseases accompanied by erythroblastosis are probably the result of an adjunction of multiple allelomorphs to the normal gene or genes for the formation of blood in the fetus. The following considerations, however, tend to invalidate such conclusions:

(*a*) Macklin apparently did not distinguish between idiopathic anemia of the new-born with erythroblastosis and Cooley's erythroblastosis; nor is it clear that she differentiated hereditary acholuric jaundice from familial icterus gravis of the new-born, since she included in her discussion Hichens'[42] cases of 2 children who, with their mother and other siblings, seem to have suffered from hereditary hemolytic icterus.

(*b*) The premise that the gene or genes for blood formation in the fetus are involved in these diseases is open to question, since there are cases on record, as already pointed out, in which abnormal extramedullary hematopoiesis was demonstrable neither by the finding of erythroblastemia during life nor by the observation of erythroblastosis at necropsy, while on the other hand, erythroblastosis is not uncommonly found in infants with congenital syphilis.

(*c*) If these diseases were dependent on a dominant mutation occurring in the germ plasm of one of the parents, they would be distributed at random among the offspring, since it would be largely a matter of chance whether any one child inherited the modified gene or not. In the familial conditions under discussion, however, the distribution is not random. The normal children are born first, while following the birth of the first affected child one or another of these conditions occurs in every child born subsequently by the same mother. The severity of the condition may vary somewhat, and occasionally the manifestation of the disease may be so mild that spontaneous recovery is possible, as remarked by Hawksley and Lightwood,[1] but a palpable liver and spleen and anemia, however benign, may still indicate the familial abnormality.[27]

(*d*) The deductions and additions with which Macklin weights her data bring the incidence of each of the related disorders fairly close to 50 per cent, but on critical examination such procedures seem unjustifiable. As various authors have pointed out,[23] it is probable that many of the miscarriages and stillbirths in families with familial icterus gravis neonatorum, as well as in those affected with fetal hydrops, have an etiology identical with that of the condition affecting the infants who survive birth or longer. When aborted fetuses and stillborn infants are included with the infants manifesting one or the other of these conditions, the incidence of the respective diseases in affected families is increased, according to Macklin's own figures, as shown in the following tabulation:

	Incidence with Exclusion of Abortions and Stillbirths	Incidence with Inclusion of Abortions and Stillbirths
Fetal hydrops	30.1%	55.3%
Icterus gravis neonatorum.....	58.1%	70.0%

42. Hichens, P. S.: Proc. Roy. Soc. Med. (pt. 1) **6**:197, 1913.

Furthermore, it must be considered that fetal hydrops may be manifested in a family affected with icterus gravis neonatorum, and icterus gravis may be seen in a child of a family usually affected with congenital anemia of the new-born, as in Diamond, Blackfan and Baty's case, cited; or, rarely, anemia and hydrops may occur in the same family. Thus, while one or another of these conditions does predominate in any one family, the incidence of affected children in a family cannot be computed on the basis of one only of these three diseases.

(*e*) The extent to which the afflicted parents may have limited their offspring when they became aware of the repetition of the malady in each new child may be only speculated on. The effect of such limitation, however, may be estimated from Macklin's data: In 17 families of 3 children each there were 26 affected children, an incidence of 50 per cent; but in 2 families of 15 children each there were 27 affected children, an incidence of 90 per cent. This difference suggests that the potential incidence in affected families might well be given more consideration in formulating hypotheses as to the inheritance of these diseases.

(*f*) The assumption that inheritance of a dominant mutation is indicated by an incidence of 50 per cent in affected families and the conclusions from her data as interpreted by Macklin may be questioned further. Dr. Dorothy Adkins, of the University of Chicago, has supplied a critical evaluation of Macklin's statistical treatment of her data and the conclusions therefrom.[43] She has pointed out that if a mutation involves the germ cell ancestral to all subsequently formed germ cells (by division) in one of the parents, the incidence of affected offspring would approximate 50 per cent, as postulated by Macklin. Such a mutation involving blood formation, however, would be extremely rare. If, on the other hand, mutation occurs in a gene in a germ cell formed by later divisions of the ancestral cell, as is much more frequently the case, the incidence of affected offspring would be something less than 50 per cent, approaching low values in some instances, according to the division at which the mutation occurred.[44] It is obvious, therefore, that the incidence of icterus gravis neonatorum at least, in a series of affected families, is far too high for a mutation in a germ cell in one parent to be responsible. This is especially true if stillborn infants, aborted fetuses and siblings manifesting fetal hydrops or congenital idiopathic anemia are included, an inclusion which seems not only justifiable but

43. Personal communication to the author.

44. This opinion was corroborated by Dr. Sewall Wright in a personal communication on the subject to Dr. Adkins.

required. She has pointed out further that if a formula is used which disregards hereditary transmission and is based only on two arbitrary assumptions, namely, (1) that once a child is born with one of these conditions (icterus gravis neonatorum, for example) all subsequent children in that family will be affected, and (2) that each child in a family of affected children is equally likely to be the first child affected, the predicted number of affected children in a large number of families agrees more closely with the actual number of affected children than does Macklin's prediction based on the postulation of a dominant mutation.[45]

This greater agreement with the actual incidence (391 calculated, compared with 394 actually affected) is obtained with no assumption as to a hereditary mode of transmission. Thus Macklin's thesis of a dominant mutation does not derive necessarily from her statistics. On the contrary, the relatively high incidence of these related conditions in the children of affected families suggests that the conditions are inherited neither as a recessive characteristic nor as a dominant mutation.

It may be maintained that in these disorders the defective anlage is responsible not so much for a fetal maldevelopment as for a delay in maturation which precludes adequate function of certain organs, chiefly, for example, the liver, thereby rendering the infant at birth unfit for extra-uterine life. If this is the case, one has no explanation of those cases of anemia of the new-born in which the anemia comes

45. Thus, for example, utilizing Macklin's statistics on icterus gravis neonatorum (table 1) and applying the formula $\frac{n(n+1)}{2n^2}$ or $\frac{n+1}{2n}$ one has:

Children in Family*	Families	Total Number of Children	Total Number of Children Affected	Children Affected, R† $\frac{¼}{1-(¾)^n}$	Children Affected, D‡ $\frac{½}{1-(½)^n}$	Children Affected, Calc. (Adkins) $\frac{n+1}{2n}$
1	7	7	7	7.0	7.0	7.0
2	25	50	32	28.5	33.3	37.5
3	17	51	26	22.0	29.1	34.0
4	23	92	58	33.6	49.0	57.5
5	20	100	62	32.7	51.6	60.0
6	13	78	37	23.7	39.6	45.5
7	4	28	15	8.0	14.1	16.0
8	8	64	36	17.7	32.1	36.0
9	9	81	46	21.8	40.5	45.0
10	4	40	30	10.6	20.0	22.0
11	1	11	10	2.8	5.5	6.0
15	2	30	27	7.5	15.0	16.0
16	1	16	8	4.0	8.0	8.5
	134	648	394	219.9	344.8	391.0

* All miscarriages and stillbirths were omitted when the number of children in the family was computed, and all children in whom severe jaundice developed were included with the affected children.

† R indicates the theoretical number of affected children if the condition is inherited and dependent on a single recessive gene substitution.

‡ D indicates the theoretical number of affected children if the condition is inherited and dependent on a dominant mutation in the germ plasm of one of the parents.

on suddenly from seven to fifteen days after birth and in which the symptoms of toxicity or abnormality appear only with the onset of the marked pallor. Segar and Stoeffler,[46] for example, reported the case of a child whose blood counts up to the seventh day showed a fall in hemoglobin and red cells "which was scarcely appreciable." Two transfusions on the eleventh and seventeenth days were followed by a remission of symptoms, but during the fourth week the anemia increased, projectile vomiting recurred, and two more transfusions were necessary. Why, it may be asked, was the liver apparently adequate for the first seven days after birth, to become inadequate until after the fourth week? This delayed appearance of symptoms in a disorder which seems to be intimately related etiologically to icterus gravis neonatorum simulates rather a sort of "incubation period." Moreover, the livers both of full term and premature normal infants may be frequently considered inadequate, as indicated by dye excretion tests, probably by reason of a certain immaturity,[36] yet such immaturity is by no means incompatible with life, even when there is resulting jaundice (physiologic). The symptoms in these diseases of the new-born characterized by erythroblastosis seem to resemble closely those of severe intoxication. Just how a defect of the germ plasm, whether it is manifested in excessive extramedullary hematopoiesis or in hepatic immaturity, is responsible for such symptoms remains obscure. As the theory stands, the strongest and practically the only argument in its favor is the fact of the familial repetition, and this can be more fully accounted for by a different mechanism. Furthermore, as de Lange [23] pointed out, "in those cases of icterus gravis neonatorum in which recovery occurs there is no further evidence of a defective anlage of the hematopoietic system."

3. Diamond, Blackfan and Baty [20] were also impressed by the pronounced extramedullary erythropoiesis and have set forth the view that a metabolic disturbance of the hematopoietic system is the underlying cause of the conditions found in the three associated diseases, edema, icterus gravis and anemia of the new-born, resulting directly in the increased extramedullary hematopoiesis, which in turn gives rise to an overgrowth of immature erythrocytes. This increased extramedullary activity is not called forth, in their opinion, by increased hemolysis, since it is greatest in a condition in which hemolysis is less in evidence, namely, in universal edema of the fetus. The increased destruction of erythrocytes also results from this metabolic disturbance, the edema resulting from the concomitant anemia. Nervous manifestations and respiratory distress are associated with the nuclear icterus in the brain. No explanation of the nature or cause of the metabolic disturbance

46. Segar, L. H., and Stoeffler, W.: J. Pediat. **1**:485, 1932.

is attempted. Actually there is no evidence that the specific antianemic factor is lacking, the absence of which causes an arrest of development at the megaloblast stage, according to Haden,[47] since most of the immature cells have gone beyond the megaloblast stage to the erythroblast and normoblast and even to the reticulocyte stage, nor has the administration of liver extract been of demonstrable value.[27] It is likewise obvious that iron is not lacking. Hence the nature of this disturbance in metabolism is quite obscure. Furthermore, while the jaundice is explained, although the condition in which the greatest hemolysis is presumed (icterus gravis) is not that in which the greatest erythroblastic activity is usually seen (fetal hydrops), it is hardly justifiable to base the appearance of edema on anoxemia due to the anemia alone, since edema is practically never seen in the relatively benign idiopathic anemia of the aregenerative type, in which the diminution of red cells is often marked. The symptoms of nervous and gastro-intestinal irritation and of respiratory distress, moreover, are only occasionally associated with nuclear icterus. The diffuse necrosis sometimes found in the liver,[19] the edema, hemorrhage and cellular infiltration in the lungs, the nuclear pathologic change in the brain which when present is associated with an icteric pigmentation differing chemically from that of ordinary bilirubin are likewise not adequately accounted for by this theory. And finally, there is no explanation for the occurrence of the same or a related condition in siblings of subsequent birth.

4. A. F. Abt [4] elaborated a theory, also based on the presence of erythroblastosis, which he termed "embryonal hematopoietic persistence." The persistence of extramedullary hematopoiesis beyond the fetal stage he ascribed to a defect in the anlage or in the germ plasm in the familial cases and to a toxic influence early in pregnancy in the sporadic case. According to his statement, "hematopoietic foci are specific for, and outstandingly characteristic of, fetal liver." These foci give rise to an overwhelming number of immature red cells, which, by reason of their immaturity, reduce the oxygen-carrying power of the blood and cause damage in the liver from anoxemia. This anoxemia may also cause the enlargement of the placenta. Since these immature cells are more fragile, they are destroyed more easily and rapidly, bringing about anemia and the formation of larger amounts of bilirubin and hemosiderin than normal. This excessive production of bilirubin, which, he stated, is merely an intensification of the destruction of hemoglobin which normally occurs in the new-born period, causes the formation of bile thrombi in the biliary capillaries, with resulting diffusion of the bile first into the lymph and then into the blood. This regurgitation of bile increases the jaundice already present from the retention of bili-

47. Haden, R. L.: J. A. M. A. **104**:706, 1935.

rubin due to hemolysis and explains the positive direct, in addition to the positive indirect, van den Bergh reaction. The bile is then excreted through the kidneys, giving rise to the "beer-brown" amniotic fluid and to the golden yellow vernix caseosa. This explanation, according to Abt, does not need "to assume a primary metabolic disturbance of the entire hematopoietic system, or a compensatory origin of the extramedullary islands." He stated, however, that further theoretic considerations "must commence with the cause for the persistence of the embryonal type of blood formation."

While this hypothesis has the merit of being explicit, one of its weaknesses is the assumption of premises on which there is no universal agreement. The assumed greater destructibility of immature erythrocytes within the body is in point. Another assumption which must be questioned is that relating to the dilated, bile-filled canaliculi, which are interpreted as "bile thrombi." As Rich [36] stated:

> There are, undoubtedly, instances of febrile infections associated with increased red-cell destruction in which one finds many canaliculi distended with bile at autopsy, but one cannot at once accept these distended canaliculi as being plugged with bile thrombi. It is interesting that such distended canaliculi are ordinarily found in the central part of the lobule about the efferent vein, a fact which suggests a relationship to a zonal damage of liver cells rather than to obstruction because of a general inspissation of the bile. It is possible, it seems to me, that the accumulation of bile in these centrally situated canaliculi may be merely a result of the depression of function of the central cells to the point that they are unable to excrete and force the bile under pressure through the canaliculi. . . . As a matter of fact just this over-filling of the central canaliculi can often be seen in cases in which a poison or chronic passive congestion has damaged specifically the central cells.

The distention of the biliary capillaries with bile, therefore, might justifiably be construed as an effect due to the same process which causes injury to the parenchymatous cells. Furthermore, dilated bile canaliculi are not invariably observed in histologic sections of the liver in erythroblastosis. Diamond, Blackfan and Baty,[20] for example, reported their absence in 2 cases, and de Lange [23] likewise noted their absence in a case of hers. It is possible that a backing up of bile due to sluggish excretion is a factor in the production of a positive direct van den Bergh reaction, but the fact must not be ignored that necrosis of liver cells is frequently observed histologically, and such injury must be considered as a further and perhaps more important reason for the positive direct van den Bergh reaction. Abt's explanation of this injury of the liver as due to the anoxemia resulting from the inadequate oxygen-carrying power of immature erythrocytes will be considered later. The symptoms of cerebral and gastro-intestinal irritation, however, are adequately explained, and, as Abt himself says, the erythroblastosis is still unaccounted for.

A further criticism of the view that erythroblastosis is responsible for the clinical and pathologic manifestations in edema, icterus gravis and anemia of the new-born, was expressed by Parsons, Hawksley and Gittens.[48] They pointed out that "in some cases of fetal hydrops such foci (erythroblastosis) are present only to a very limited extent or may be absent," as noted in the case studied by them, "thus providing strong evidence that they do not represent the origin of these disorders." They felt that the erythroblastosis is only "a concomitant feature or response . . . to an increased call for erythropoiesis" and that "this response is therefore a symptom and not a cause of the disease." The even greater inconstancy in degree, in fact occasionally the almost entire absence, of extramedullary hematopoiesis or erythroblastemia in icterus gravis and in congenital anemia of the new-born suggests strongly that erythroblastosis, while it usually accompanies, is by no means the fundamental cause of, this group of familially related diseases.

5. Knoepfelmacher,[49] Beneke [50] and Pfältzer,[51] among others, were convinced on the basis of their positive bacteriologic findings that icterus gravis neonatorum is essentially a septicemia. Pfältzer suspected Bacillus coli or organisms closely resembling it, as the most likely agents, since he found clumps of such bacilli between fibers of skeletal muscles. Both the umbilical stump and the erosions of the digestive tract known as "stigmata ventriculi" have been suggested as possible portals of entry. Zimmerman and Yannet,[14] on the basis of Dunham's [52] studies of jaundice and sepsis in the new-born, declared unequivocally for sepsis as the underlying etiologic factor in icterus gravis neonatorum. Grulee [53] felt likewise that "in all probability icterus gravis neonatorum is associated with infection, at least in the vast majority of cases." I. A. Abt,[3] however, was satisfied both from clinical observation and from bacteriologic study that in his cases the condition was not due to sepsis, and Hawksley and Lightwood [1] felt also that the evidence did not point that way. Yllpö,[40] because of negative blood cultures for both aerobic and anaerobic organisms, his extraordinary care to preserve asepsis, the early appearance of the jaundice (one and a half hours after birth), the increased bilirubin content in blood from the cord at birth and the absence of bacteria in histologic sections of the various organs, asserted that postnatal infection cannot be presumed to

48. Parsons, L. G.; Hawksley, J. C., and Gittens, R.: Arch. Dis. Childhood **8**:159, 1933.

49. Knoepfelmacher, W.: Ergebn. d. inn. Med. u. Kinderh. **5**:205, 1910.

50. Beneke: München. med. Wchnschr. **59**:387, 1912.

51. Pfältzer, B.: Ztschr. f. Geburtsh. u. Gynäk. **76**:685, 1914-1915.

52. Dunham, E.: (*a*) Am. J. Dis. Child. **40**:671, 1930; (*b*) **45**:229, 1933.

53. Grulee, C. G., and Bonar, B. E.: The Newborn: Physiology and Care, New York, D. Appleton and Company, 1926, vol. 2.

be responsible. Palm [54] stated likewise "that a new-born infant who . . . having come into the world under all aseptic precautions, sickens a few hours later with a jaundice becoming ever more intense hour by hour . . . dies without clinical as well as without pathologic or bacteriologic evidence on necropsy of the slightest trace of infection either of the navel or of the gastro-intestinal tract renders it unlikely that sepsis is being dealt with." Klemperer,[19] too, considered that the diffuse necrosis to be noted in his first case, in particular, spoke against infection in which the necrosis is usually focal. Dunham's studies certainly indicate the necessity for extremely careful and thorough investigation of all cases of jaundice in the new-born, but they do not enlighten one as to the cause of the familial type of icterus gravis of the new-born in cases in which healthy parents, a negative prenatal history and entirely negative bacteriologic findings fail to justify the assumption of infection. Severe jaundice secondary to sepsis must not be placed in the same category with the disease under discussion, and a careful differentiation must be made between those cases in which an infection is superimposed on the primary pathologic process and those in which the infection itself is primary. It seems to be rightly conceded that sepsis, although its symptoms may exactly simulate those of icterus gravis neonatorum, may be excluded as a fundamental etiologic factor in this disease. This opinion is strengthened by the fact that much of the bacteriologic work done in this condition has yielded negative results.[55]

6. Hampson,[7] in considering possible factors causing physiologic jaundice of the new-born, formulated two tentative explanations for the increase in blood bilirubin after birth: 1. Before birth bilirubin may be excreted through the placenta into the maternal blood stream; after birth the liver may be some time in assuming its full excretory function, and hence bilirubin may accumulate in the infant's blood. 2. Some maternal influence may be assumed, possibly hormonic, which may restrain hemolysis; after birth, if this factor is temporarily absent or deficient in the child, hemolysis, owing to alterations in oxygen tension, may become excessive, with the production of jaundice. Hampson held that icterus gravis neonatorum closely resembles physiologic jaundice of the new-born and can be explained by presuming an even greater delay in the assumption of hepatic function than in the latter condition. He stated, however, that "whether the delay in assumption be one of excretion, or in the production of an internal secretion, it seems probable that it is one in which the liver is largely concerned." Hampson felt that his record of 17 recoveries in 18 infants who were

54. Palm, H.: Monatschr. f. Geburtsh. u. Gynäk. **49**:264, 1919.

55. Abt.[3] Diamond and others.[20] Yllpö.[40] Palm.[54]

treated with small intramuscular injections (5 to 15 cc.) of mother's serum daily tended to substantiate his second hypothesis. Hawksley and Lightwood,[1] however, called attention to the fact that anemia frequently continues to develop rapidly even after transfusions, a procedure which would introduce several times as much serum into the vein as Hampson injected into the muscles. Furthermore, they found intramuscular injections of whole blood of no benefit in the infants with erythroblastemia and used human blood serum without success. They felt "that the transfused blood acts by substitution and not by supplying any hypothetical anti-hemolytic substance." On the other hand, their implied explanation of Hampson's success as due to spontaneous recovery is not warranted by the statistical evidence of 80 per cent mortality in untreated patients. It seems that small amounts of the serum of the child's mother must have some value when the disease is mild, but it also seems obvious that the explanation is not that set forth by Hampson. His alternative hypothesis, however, that bilirubin may increase in the infant's blood after birth because of hepatic insufficiency, while before birth the excess pigment is excreted through the placenta, seems to be borne out by Yllpö's observation of an elevated icteric index in blood from the cord, although the jaundice did not become apparent until one and a half hours after birth.

Hampson further suggested that the products of the abnormal hemolysis of red cells may be toxic and give rise to the symptoms of the disease. But the destruction of erythrocytes must itself be secondary to a more primary process still unknown; nor is the familial tendency in these diseases explained by such an assumption.

7. Parsons, Hawksley and Gittens,[48] among others, felt that some toxic factor must be the responsible agent in this disease. De Lange and Arntzenius [18] expressed this possibility as follows:

> There are observations of liver-cell necrosis in the literature . . . and such findings show a certain agreement with acute or subacute yellow atrophy of the liver, the occurrence of which is generally associated with the action of a chemical poison. Chemical poisons produced by the mother or by the child itself can cause hemolysis in the child during pregnancy. This destruction of blood gives rise to increased formation of blood . . . which finds its anatomic expression in the changes described in the organs.

This theory appeals by reason of its simplicity. It explains both the symptoms and the pathologic changes. It is easily conceivable that a toxic influence is at work for some time before birth (there seems to be evidence for this in fetal hydrops) and that the mother's circulation is the mechanism by which the child is spared in utero. When the cord is severed and this avenue for the elimination of toxic substances is cut off, the poisonous products may accumulate, causing increased destruction of erythrocytes, and jaundice, if not already

present, may appear. This would explain why the child may appear vigorous at birth and yet suffer a subsequent rapidly fatal decline. The same mechanism may operate to prevent an accumulation of bilirubin in the child in utero, as suggested by Hampson. If in utero the pathologic process becomes fulminating, the toxic substances may not be removed rapidly enough, and edema, jaundice and numerous toxic changes in the organs may result, as in fetal hydrops, or they may even cause the intra-uterine death of the fetus. This is essentially the very plausible explanation offered by Palm.[54]

But an impasse is reached when an explanation of the nature or the source of such a toxic influence is attempted. It is true that in cases too numerous for the observation to be ascribed to chance alone the mother may have various abnormal symptoms, and for this reason Hoffmann and Hausmann [6] and others felt that toxic substances resulting from the pregnancy may affect the fetus adversely. They pointed out that in cases in which the mother is free from symptoms the intoxication may be insufficient to affect the mother clinically, while the more sensitive fetus is affected harmfully. The fact must be reiterated, however, that in the majority of cases of icterus gravis neonatorum the mother reveals no symptoms which might give a clue to the source within herself of a possible toxic substance. The blood pressure of the mother during pregnancy in most of these cases is low rather than high; evidence of renal injury is slight or entirely lacking, and carefully supervised prenatal observation and care, as in Smyth's [31] and in Abbott and Abbott's [27] cases, may reveal nothing abnormal. On the other hand, the incidence of maternal symptoms is much more frequent when the pregnancy results in a child with fetal hydrops. As may be noted in the report of Bullard and Plaut [33] cited, the mother had no symptoms during her second and third pregnancies, although the infants presented icterus gravis neonatorum, but during the fourth pregnancy the symptoms were severe, and the infant was born hydropic. There appears to be some interrelationship, therefore, between the condition of the mother and that of the fetus, at least while the fetus is alive,[35] with the suggestion of a parallel in the severity of the symptoms in each, but it is impossible with the evidence at hand to be certain whether the condition of the mother or that of the child is primarily responsible. The fact that the mother is the outstanding constant factor in the manifestation of the disease in a series of offspring, however, must be borne in mind.

Von Reuss [56] suggested that possibly toxic substances derived from the intestinal tract, formed as the result of bacterial decompositon,

56. von Reuss, A.: Die Krankheiten des Neugeborenen, Berlin, Julius Springer, 1914.

might be responsible for the symptoms of intoxication in the icteric infant. This possibility, however, seems remote when one considers Yllpö's [40] case, in which the infant became icteric one and a half hours after birth though every aseptic precaution was taken and though nothing had been allowed to enter the infant by mouth. Nor would such toxic substances explain a stillborn edematous fetus.

Hoffmann and Hausmann [6] presented the idea that a food allergy, although not the fundamental cause, might be the ground for exacerbation of the disease after birth. They reported the case of an infant in whom marked gastro-intestinal symptoms developed when it was given its own mother's milk, although it could take a strange woman's milk with impunity. A slightly older normal infant was likewise affected when given this mother's milk. This mother had had several children afflicted presumably with familial icterus gravis neonatorum. The child she did not suckle had only slight jaundice. This recalls Rolleston's [32] admonition to adhere to Auden's policy of not permitting affected infants to take their own mothers' milk. The possibility that the milk of the mother of an affected child might be responsible for the exacerbations following remissions, such as were noted in the case reported by Segar and Stoeffler [46] cited, has not been seriously considered, since there are no outstanding data to verify such an assumption, nor is there as yet a rational explanation for any such effect. Nevertheless, in view of the interesting observation of Hoffmann and Hausmann in this connection, such a possibility might well be investigated.

A number of observers have with considerable justification ascribed many of the symptoms and pathologic changes found in this disease to liver dysfunction.[57] These symptoms, apart from those referable to the blood cells, show a striking similarity to those resulting from poisoning by alkaloids affecting the parasympathetic system. Other substances having the same effect are those resulting from tissue breakdown, such as choline, histamine, guanidine, the proteoses and peptones. Such substances may well be derived from the injured liver of the child itself. Excreted into the maternal circulation through the placenta in sufficient amounts, as might occur in fetal hydrops, for example, the fetus may become a *Giftquelle* for the mother, as suggested by Palm.[54] The maternal symptoms might then indeed be such as have been noted here from the literature. Further evidence implicating the fetal liver will be considered later. That toxic substances resulting from such dysfunction or injury in this organ must in turn be explained by a more fundamental pathologic process, however, is obvious, and again the familial tendency must still be accounted for.

57. Hoffmann and Hausmann.[6] Pfannenstiel.[39] Palm.[54]

A further question may be raised as to whether the anemia or the destruction of blood really gives rise to compensatory regeneration so extensive as that found in erythroblastosis. Certainly this is not the case in congenital anemia of the nonregenerative type.[27] Furthermore, as Gierke [16] pointed out, the anemia in icterus gravis neonatorum may in occasional cases be slight or absent, while the erythroblastosis in such cases may be marked. It seems, therefore, that as a compensatory effort secondary to destruction of blood or to anemia alone the erythroblastosis is inadequately explained.

8. Woolley [34] speculated on the erythroblastosis found in congenital edema (hydrops), and since the evidence is in favor of an etiologic relationship between this condition and icterus gravis neonatorum it may be well, for the sake of completeness, to consider it in this study. He suggested that there was "something of an analogy between erythroblastosis and leucemia" and that erythroblastosis might also be looked on as belonging to the group of tumors, in this case the erythroblastic tissue being involved. Salomonsen [17] also tentatively considered this possibility. This hypothesis was set forth by Woolley in 1916, before the familial association between fetal hydrops and icterus gravis neonatorum was recognized. Since it has been found that icterus gravis neonatorum, even in cases in which there is marked erythroblastemia, responds in a fair number of instances to early and adequate blood transfusions with eventual and lasting recovery, erythroblastosis neonatorum can scarcely be conceived as a type of new growth of erythroblastic tissue comparable to that involving leukoblastic tissue in leukemia.

It appears that no theory has yet been presented which accounts for icterus gravis neonatorum and the apparently allied conditions in an entirely adequate fashion. The erythroblastosis has greatly intrigued those studying these diseases, but the variability of this factor suggests that its importance as a primary etiologic influence has been overemphasized. It seems more probable that it is rather a symptom, in the sense in which the jaundice, the leukocytosis and the hemorrhagic tendency are symptoms, of a more fundamental underlying pathologic process which has not yet been determined. The frequency of its appearance in those cases which come to necropsy suggests that it is associated with the more severe forms of that pathologic process.

The familial association of edema, icterus gravis and idiopathic anemia of the new-born, however, permits one to draw certain tentative conclusions which might not otherwise be justifiable. If, for example, one assumes that these three conditions are etiologically related, one cannot ascribe the evidence of greater injury in the liver, brain and other organs in icterus gravis neonatorum to anemia alone, since in

congenital anemia, especially of the nonregenerative type, the anemia is often far more intense, while the evidence of injury to the organs is less outstanding and spontaneous recovery relatively frequent. Furthermore, if, as A. F. Abt [4] postulated, the immaturity of the red cells is the cause of the damage in the liver, owing to the fact that immature erythrocytes are less effective carriers of oxygen than mature cells, it is not apparent why, in those cases of idiopathic anemia of the newborn in which erythroblastemia is not evident in the beginning, the appearance of young red cells is coincident with the onset of definite recovery, as in the case of Abbott and Abbott [27] cited. The observations of Rich and Resnik as to the effects of oxygen deficiency on the liver parenchyma caused Snell [58] to investigate the question whether anoxemia is ever found in patients with clinical hepatic disease, and as a result it was found that anoxemia of the anoxic variety was a fairly constant accompaniment of advanced parenchymal hepatic disease, with some evidence to show that the degree of oxygen unsaturation of the arterial blood in general reflected to some extent the degree of hepatic insufficiency. One must therefore consider that the damage of the liver may not be so much a result of anoxemia as a cause of it in the conditions under analysis. It is true, however, as Judd, Snell and Hoerner [58] pointed out, that while it is difficult to determine definitely the true cause of the anoxemia sometimes observed in cases of hepatic disease, it is quite likely that it in turn has some effect on the progress of the hepatic lesion. It may be seen that one can be easily confused as to what is cause and what is effect. Are the bile thrombi the primary cause of the regurgitation jaundice, or are they, as Rich's statement suggests, the result of damage to the parenchyma? Is the damage in the liver entirely the result of anoxemia of either the anoxic or the anemic type, or is the anoxemia due, at least in part, to hepatic insufficiency? Judd, Snell and Hoerner felt that possibly the low percentage of oxygenation of the arterial blood in patients whose livers have been damaged may be attributable to some change in the pulmonary alveoli causing deficient oxygenation of the reduced hemoglobin, since inhalation of oxygen materially increases the oxygen saturation of the arterial blood. They called attention to the work of Rühl, who observed minute changes in the alveolar walls in experimental animals after shock due to histamine. It seems to be obvious that the answers to the stated questions and the solution of the problems implied are of definite importance for the more complete understanding of icterus gravis neonatorum.

58. Judd, E. S.; Snell, A. M., and Hoerner, M. T.: J. A. M. A. **105**:1653, 1935.

It must be repeated that some observers have found no necrosis in the histologic sections of livers from infants dying of this disease.[59] Is one to assume that in such cases hepatic insufficiency plays no part? The answer must be sought in the clinical manifestations of the disease. It has been thought that the jaundice plays a prominent role in the tendency to hemorrhage.[2] But the assumption that bile pigment is itself capable of causing hemorrhage has been disputed. Scott [60] stated that there is no relation between the degree of jaundice and the tendency to bleed. He reported operating on a baby with an icteric index of 320 and finding no tendency to hemorrhage from either the muscle or the bed of the spleen. Kass and Osgood [41] also found no tendency to bleed in an infant receiving a diet high in carbohydrate to sustain the liver, who lived almost a year with complete obliteration of the extrahepatic bile ducts. Furthermore, in hereditary hemolytic icterus there is slight, if any, tendency to bleed. Judd and his collaborators stated that bleeding attributable to hepatic diseases has been known to take place in the absence of clinical jaundice. They suggested that the degree of injury to the hepatic parenchyma may be the most important factor in the production of hemorrhage in cases of hepatic disease. Ottenberg [61] likewise held that "the tendency to bleed depends rather on the extent of liver parenchyma damage than on the jaundice itself." It is entirely reasonable to assume, therefore, that injury of the liver may play a dominant part in the production of the "hemorrhagic diathesis" found in the conditions characterized by erythroblastosis, since it is in those conditions that the purpuric manifestations may occur. In the case of the fifth child of family B, reported by de Lange and Arntzenius,[18] there were hemorrhages in the skin and a conjunctival hemorrhage in the right eye, but in microscopic examination of the liver on necropsy only slight fatty changes were found, with no necrosis. It may not be assumed unquestioningly that absence of histologic evidence of necrosis in the liver necessarily rules out injury of the liver. That this organ is fundamentally involved in icterus gravis neonatorum is not a new concept, of course. I. A. Abt,[62] for example, definitely expressed the belief that "an anatomic inferiority of the liver" is responsible for the symptoms of the disease.

Gierke's [16] report likewise gave interesting corroboration to the view that hepatic damage is a factor in these conditions. He sent a sample of the red-brown serum from the bloody effusion found in the

59. Huwer, G.: Ztschr. f. Geburtsh. u. Gynäk. **94**:150, 1929. de Lange and Arntzenius.[18]

60. Scott, J. M., in discussion on Judd, Snell and Hoerner.[58]

61. Ottenberg, R.: J. A. M. A. **104**:1681, 1935.

62. Abt, I. A., in discussion on Dunham.[52a]

peritoneal cavity in his case, which reacted negatively to the Gmelin test, to Thannhauser, who reported as follows:

> The serum contains bilirubin, and the indirect diazo reaction is stronger than the direct. In addition to bilirubin, the pigment designated by us as xantorubin, which we have found in dogs after extirpation of the liver, is also demonstrable spectroscopically. . . . I believe that this finding indicates a severe insufficiency of the liver.

In this case occasional atrophy of the liver cells, in addition to the presence of pigment, was the only observation made regarding the hepatic parenchyma itself.

Gierke felt that this finding was of especial interest in view of the fact that the nuclear staining in nuclear icterus seems to be brought about by a pigment other than bilirubin, as pointed out by Schmorl,[13] since in solution of formaldehyde and solutions of mercuric chloride it does not show the green color characteristic of the oxidized bilirubin. The nuclear staining with necrosis of the ganglion cells found in the brain in some cases of this disease has been related to hepatic injury by a number of observers. Hoffmann and Hausmann,[6] for example, expressed the belief that these changes are due not to an excess of bile pigment, since cerebral symptoms are never met with in the severe cholemia which accompanies congenital atresia of the bile ducts, but to some substance or substances, possibly lipolytic, produced by the necrosis of hepatic tissue. They pointed out that cerebral necrosis does not occur without hepatitis in those diseases in which the liver and brain are both affected. Wilson[63] himself was impressed by the analogy between icterus gravis neonatorum showing nuclear changes and the disease of young adults associated with cirrhosis of the liver described by him under the term "progressive lenticular degeneration." Crandall and Weil,[64] furthermore, showed experimentally that changes in the brain may be related to injury of the liver. They demonstrated in the serum of dogs, as early as the fourth day following ligation of the common bile duct, toxic substances which still exerted their destructive effects on the spinal cords of rats in vitro after being maintained at 62 C. for thirty minutes and after removal of the proteins by coagulation with acetic acid and boiling. They found also that these toxic substances are not identical with the lipases, which are increased simultaneously. The insult to the liver was followed both in dogs and in rats by spongy necrosis of nerve tissue in the brain, active proliferation of glia and a diffuse disease of nerve cells with predilection for the deep cortical layers and the striate body. In rats vascular changes were more marked than in dogs, and the degeneration of nerve cells was more pronounced in the basal ganglion, the

63. Wilson, S. A. K.: Brain **34**:296, 1912.
64. Crandall, L. A., and Weil, A.: Arch. Neurol. & Psychiat. **29**:1066, 1933.

midbrain and the cerebellar nuclei and less in the cortical cells. All the experimental animals, on histologic examination of the liver, revealed severe degenerative changes of the hepatic cells.

Judd, Snell and Hoerner [58] emphasized the value of repeated transfusions of blood, as well as of the use of oxygen in the treatment of hepatic disease to relieve the anoxemia whether of the anoxic or of the anemic variety, and stated that more benefit is apparently received from a transfusion than can be attributed to the amount of hemoglobin alone. The great value of intravenous transfusions of sufficient quantities of blood in the treatment of icterus gravis neonatorum is demonstrated in numerous case histories in the literature. The often dramatic recovery of some of the infants after repeated transfusions, with no subsequent evidence of developmental anomaly provided the brain has not been unalterably injured, suggests that the liver is probably the chief beneficiary, strengthening the impression arrived at by observation and analysis of the clinical and pathologic manifestations that a pathologic insufficiency does exist in the liver, not due to a "constitutional perversion" but to a definite injury or diseased state. It also renders untenable the theory that this condition of the liver is due to a defective anlage or to defective germ plasm.

It is now profitable to consider these conditions of the new-born, in which injury of the liver may be presumed to be indicated and in which one very often observes erythroblastosis, in contrast to a condition in which no such indications are found. If damage to the liver is slight or has not occurred and abnormal destruction of erythrocytes takes place alone, there may presumably be only slight transient jaundice or none, since the undamaged liver could excrete the excess of bilirubin. Edema and the tendency to bleed will also be absent, and although the anemia may become extremely severe, the process will be relatively benign and spontaneous recovery relatively frequent. This seems to explain the clinical manifestations of congenital anemia of the nonregenerative type. The relative freedom of the liver from damage in this condition is further suggested by Rich's [36] statement, that "whether or not jaundice will occur in any case depends upon the balance between the amount of bilirubin delivered to the liver for excretion and the capacity of the liver to excrete the pigment . . . with very rare exceptions the development of jaundice, even in the absence of obstruction, depends at least partly upon a lowering of the excretory ability of the liver."

In nonregenerative congenital anemia, then, one has reason to suspect that the liver is implicated only slightly or not at all, while in the diseases in which erythroblastosis is a feature, which are frequently fatal when untreated, the clinical evidence of hepatic dysfunction is

strong. Whether the erythroblastosis is a response to the anoxic anoxemia resulting from injury to the liver, since no such response occurs in the aregenerative anemia of the new-born in which the anemia may be severe, remains a question, but there is good evidence for it. De Lange [18] felt that transfusions are not indicated as long as severe jaundice and marked regeneration of blood are still apparent, that only the succeeding anemia justifies this treatment. But in their baby "G," who received 40 cc. plus a further indefinite amount of the father's blood through the longitudinal sinus on the fourth day, the normoblasts and megaloblasts, which had numbered 124 per hundred white blood cells, disappeared quickly, the jaundice decreased, the spleen became no longer palpable and the liver of normal size, although the anemia continued to increase in severity. It is difficult to escape the conviction that in all probability it was the hepatic parenchyma rather than the bone marrow which benefited from the therapy and that the diminution of the erythroblastic activity resulted from the improvement in hepatic function. "Embryonal hematopoietic persistence" may thus quite possibly depend on the liver, not because the liver retains its fetal characteristics by reason of a defective anlage or defective germ plasm, but because injury to this organ has resulted in an increase of anoxemia of the anoxic type. Indeed, the resulting inadequacy of oxygenation may thus give rise to the "metabolic disturbance of erythropoiesis" postulated by Diamond, Blackfan and Baty as the cause of erythroblastosis. This idea was likewise expressed by Hoffmann and Hausmann: [6]

> It is not inconceivable that, as phosphorus through attachment to oxygen interferes with the oxidative processes in the body and thereby leads to acid intoxication, similar interferences with metabolism in fetal life could be responsible for icterus gravis neonatorum. The increase in nucleated erythrocytes and the persistence of extramedullary foci of hematopoiesis would then be conceived of as compensatory phenomena.

Eastman [65] showed that the mean capillary unsaturation for oxygen in normal fetal blood at birth is three times that of adult blood and that in utero it is twice as great. This affords an explanation of Lippman's [66] studies in which normoblasts and other young forms of blood cells were found almost invariably during the first day of life, disappearing rapidly until no longer present toward the end of the first week. This view appears to be further substantiated by the fact that Barcroft [67] found a considerable increase in reticulocytes in adults subjected to a rarefied atmosphere. The fact that nucleated forms of red cells are

65. Eastman, N. J.: Bull. Johns Hopkins Hosp. **47**:221, 1930.
66. Lippman, H. S.: Am. J. Dis. Child. **27**:473, 1924.
67. Barcroft, J., and others: Proc. Roy. Soc. Med. **16**:58, 1922-1923.

not usually found in adults under conditions of low oxygen tension is no proof that they may not occur, along with extramedullary hematopoiesis, as a response to an abnormal degree of anoxemia in the fetal and new-born period, when the lability of the hematopoietic system is marked. Suffice it to say, however, that in the aregenerative form of congenital idiopathic anemia the primary cause of the malady cannot be related to erythroblastosis.

From this review of case histories and etiologic concepts found in the literature, analyzed with reference to recent studies on jaundice particularly, the following conception regarding these related disorders emerges: Aregenerative idiopathic anemia of the new-born represents the mildest manifestation of a pathologic condition characterized by a rapid and often marked decrease in the red cells of the circulating blood. This form, although relatively benign, is nevertheless fundamentally related to those more severe forms of the malady associated with erythroblastosis. In the latter, edema, jaundice, cyanosis, hemorrhagic manifestations, gastro-intestinal and nervous irritability, and collapse are found to varying degrees. These symptoms are least evident in idiopathic anemia of the new-born, more pronounced in icterus gravis neonatorum and extreme in universal edema of the fetus; in universal edema, however, the development of marked jaundice is usually precluded by death. These symptoms, leading frequently to a fatal outcome, result from injury to the liver. Roughly proportional to the extent and severity of this injury, there is abnormal diminution in the oxygen content of the arterial blood in the affected child, a condition which gives rise to compensatory erythroblastemia and associated erythroblastosis.

In accordance with this conception, two pathologic processes are postulated as responsible, to varying degrees, for the symptoms and changes in the affected child. These are (1) abnormal destruction of erythrocytes and (2) injury of the liver. While the mother may be affected secondarily by the condition occurring in the fetus, she seems to be the primary source of the influence which gives rise to one or both of these abnormal processes in the fetus and new-born infant, since she is the one constant factor to be found when this pathologic state appears in a series of offspring. If this is the case, one must examine more closely the possible nature of such an influence and the modes of transmission by which it may pass from mother to fetus. The argument that the transmission occurs by way of the germ plasm appears definitely unsupported. There remains only one other means of transfer, namely, the placenta. That a toxic substance generated in the mother is transmitted through the placenta to the fetus seems unlikely in the absence of symptoms in the mother in most cases; the

reverse might conceivably be taking place when such symptoms occur. Furthermore, the infant affected by the aregenerative form of congenital anemia exhibits no symptoms of intoxication, but marked anemia only. In this form of the malady the erythrocytes alone seem to be concerned. If, now, the possible mechanisms giving rise to destruction of erythrocytes are reviewed,[68] it is found that all may be eliminated from consideration save one, the destruction of red cells by some form of immune reaction. If the destruction of red cells by the action of specific immune bodies is tentatively considered to represent the pathologic mechanism underlying this disease, one may reconstruct the etiologic events as follows: The mother is actively immunized against fetal red cells or some component of them. The immunization may conceivably occur as the result of an accident within the placenta whereby the fetal cells or their hemoglobin gain entrance to the maternal blood sinuses. The antibodies formed in the maternal organism may then pass to the child through the placenta or possibly to an even greater extent through the colostrum and milk, since the diminution of red cells in congenital anemia appears to be most acute following birth. The time elapsing before such antibodies are present in the infant in sufficient concentration to produce a marked effect may correspond to the delay in the appearance of symptoms noted in most cases of congenital anemia.[69] Such a transfer of immune bodies from an actively immunized mother to the fetus or new-born child sets up in the offspring a state of passive immunity. Such an immunity is relatively short lived and would eventually disappear completely, leaving no trace to be passed on to a succeeding generation. Furthermore, each child born and suckled subsequent to this active immunization in the mother would possess to a greater or less degree a passive immunity to the specific antigen, while any children born before the immunization of the mother would be entirely unaffected. These facts were demonstrated in guinea pigs by Anderson,[70] who showed that after active immunization of the mother a displacental transfer of specific antibodies took place in 4 successive litters; this passive immunity in the offspring, moreover, was not transmitted to the following generation. This conception of possible events leads to the formulation of a hypothesis which thus explains adequately not only the familial tendency in this disease but likewise its definite and distinctive distribution among the children of an affected family. In the operation of such a mechanism the mother would show no symptoms, yet she would transmit a destructive influ-

68. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

69. Abt.[25] Abbott and Abbott.[27] Segar and Stoeffler.[46]

70. Anderson, J. F.: J. M. Research **15**:241 and 259, 1906.

ence to successive offspring through the placenta and through her milk. This mechanism, incidentally, bears no relation to a difference in blood groups in mother and child; nor is such a difference a factor in this group of diseases.[20]

If one now reexamines the findings which are frequently encountered in so-called erythroblastosis, one finds, in addition to jaundice and erythroblastemia, (1) increased capillary permeability, manifested by edema and the "hemorrhagic diathesis;" (2) increased bleeding and coagulation times; (3) gastro-intestinal irritability, indicated by frequent stools and vomiting, suggesting vagal stimulation; (4) other effects on the central nervous system, evidenced by somnolence and flaccidity, twitchings and occasionally convulsions; (5) respiratory distress, indicated by cyanosis and "grunting" expiration, and occurrence of respiratory failure long before cessation of the heart beat, as noted by Huwer.[59] These symptoms, as suggested earlier, resemble closely those following stimulation of the parasympathetic division of the autonomic nervous system by certain drugs. Sollmann [71] included in his discussion of substances having such an action not only certain alkaloids and the toxic products of tissue autolysis mentioned previously but also a process which produces effects remarkably similar to those described here: anaphylaxis.[72] That anaphylaxis may frequently result in marked changes in the liver has been demonstrated for dogs by R. Weil,[73] particularly, and by Dean and Webb.[74] Webb [75] studied also the effects on the blood cells resulting from anaphylactic shock and found marked increase both in the nucleated forms of red cells and in the immature cells of the leukocytic series. He concluded that these were called forth from the bone marrow by the extreme anoxemia. Furthermore, while there was in these dogs immediate intense leukopenia, apparently due to extravascular migration of polymorphonuclear leukocytes, especially in the lungs—an observation which brings to mind the "bronchopneumonia" reported on necropsy in 2 cases of Diamond, Blackfan and Baty [20] and the "hemorrhagic bronchopneumonia" found in the first sibling in Abbott and Abbott's [27] first case—this was succeeded within a half hour following shock by pronounced leukocytosis with a definite shift to the left. The livers in the experimental animals of Dean and Webb were markedly congested and the sinusoids dilated.

71. Sollmann, T. H.: A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology, ed. 5, Philadelphia, W. B. Saunders Company, 1936.

72. Darrow, R. R.: Abstr., Bull. West Side Br., Chicago M. Soc. **1**:3, 1936.

73. Weil, R.: J. Immunol. **2**:525 and 571, 1917.

74. Dean, H. R., and Webb, R. A.: J. Path. & Bact. **27**:51 and 65, 1924.

75. Webb, R. A.: J. Path. & Bact. **27**:79, 1924.

In the early stages following shock and in those dogs which recovered quickly the liver cells showed cloudy swelling. Later in the more severely affected animals there were all degrees of necrosis, with practically complete disorganization of the hepatic cells in one of the animals that died. The condition of the liver in this animal, as described by the observers, was almost identical with that in the infant with fetal hydrops reported by Parsons, Hawksley and Gittens.[48] In those dogs that recovered and were later put to death, the livers were practically normal within twenty-four hours except for the increased deposits of iron pigment in all the shocked animals. Petechiae were found in the organs of many animals, and in a number hemorrhage from the gastrointestinal tract had occurred.

Without carrying the analogy further, it may be recognized that in anaphylaxis one has a mechanism which is closely related to that tentatively considered for aregenative anemia of the new-born. In erythroblastosis, however, sensitization rather than immunization seems most adequately to explain the observations. The mechanism of its transmission through the placenta and later through the milk is the same. Fetal hydrops and icterus gravis neonatorum would be accounted for by placental transmission, while the exacerbations after partial recovery noted in icterus gravis and in congenital anemia are sufficiently explained by transmission of additional sensitizing factors through the colostrum and milk. Such a hypothesis is of further interest in that it appears to explain also certain details in case histories which seem otherwise incomprehensible. Among these is Hampson's success with small amounts of the infant's own mother's serum, administered daily by intramuscular injection. If this therapy is considered as a possible desensitization of the infant, the reason for its success is evident. Wells[76] stated that antiamboceptors may be obtained by immunizing an animal with a serum which contains amboceptors, and Opie and Furth[77] found that after injecting an antigen, for example horse serum, into their animals, the animals could be desensitized to the action of antihorse serum by repeated injections of antihorse serum in quantities insufficient to cause symptoms. Rolleston's counsel to withhold the mother's milk from an affected child gains new support from this concept, and Hoffmann and Hausmann's observation on the reaction of their infant to his own mother's milk can be understood.

There is, however, one further assumption which must be made. The antigen giving rise to such amboceptors must be a protein foreign to the blood of the mother in whom the reaction occurs. The observation

76. Wells, H. G.: Chemical Aspects of Immunity, ed. 2, New York, Chemical Catalog Company, Inc., 1929.

77. Opie, E. L., and Furth, J.: J. Exper. Med. **43**:469, 1926.

of Hawksley and Lightwood [1] and the work of Trought [78] and of Barcroft [79] suggest that fetal hemoglobin may differ in its molecular structure from adult hemoglobin of the same species. If this is the case, fetal hemoglobin might be antigenic if it gained access to the maternal circulation, since Wells and Osborne [80] have demonstrated that it is the chemical character of a protein which determines its specificity rather than its biologic source. That hemoglobins are capable of acting as specific precipitinogens has, furthermore, been confirmed by Hektoen and Schulhof.[81] The assumption that fetal hemoglobin may be immunologically different from adult hemoglobin, and thus, possibly, the antigen involved, amplifies and completes the hypothesis that an antigen-antibody reaction is responsible not only for one but for all forms of this malady.

In conclusion, then, it may be proposed that in icterus gravis neonatorum and in those related diseases in which the symptoms indicate injury of the liver passive sensitization to the hemoglobin of his own red cells has been transmitted to the fetus from the mother, primarily through the placenta while the infant was in utero and through her milk after birth. In the aregenerative form of congenital anemia, on the other hand, it is likely that immunization rather than sensitization has taken place in the mother and has been transmitted as such to the child, or that, in some cases, desensitization or possibly mitigation of the sensitization reaction has in some way been accomplished.

This hypothesis is offered with due reservations, since it is based on theoretical considerations. It has been formulated as the result of a critical analysis of many case histories, with the aid of experimental studies in other fields which appeared to throw light on the pathogenesis of the disorder. It has seemed important in this review to call attention to such studies, as well as to discuss various extant hypotheses regarding the etiology of the disease, especially since they offer experimental material from which a suitable concept seems logically to develop. The proposed hypothesis differs from others considered in this paper in that it meets the criteria which were set up for their evaluation, and likewise in that it is susceptible of confirmation or refutation.

SUMMARY

Icterus gravis neonatorum is a severe disease of the new-born, characterized by jaundice appearing soon after birth, with enlargement of the liver and spleen and with clinical symptoms which may include

78. Trought, H.: Arch. Dis. Childhood **7**:259, 1932.
79. Barcroft, J.: Lancet **2**:1021, 1933.
80. Wells, H. G., and Osborne, T. B.: J. Infect. Dis. **19**:183, 1916. Wells.[76]
81. Hektoen, L., and Schulhof, K.: J. Infect. Dis. **31**:32, 1922; **33**:224, 1923.

to varying degrees any or combinations of the following: somnolence, listlessness and flaccidity, or muscular twitchings, opisthotonos and occasionally convulsions; vomiting and diarrhea; petechiae, ecchymoses and bleeding from mucous surfaces; increased bleeding and coagulation times; edema; cyanosis and respiratory difficulty; collapse. In addition are found a marked increase in the number of circulating immature red blood cells, relative anemia, and leukocytosis, with frequently eosinophilia or monocytosis or both. The child may appear strong and vigorous at birth, but the appearance of symptoms is rapid, the illness ending in death within a few hours or days, occasionally in the second or third week, in the untreated case.

At necropsy, jaundice and pallor of the tissues, bile-tinged fluid in the serous cavities and minute or more extensive hemorrhages in the lungs, brain and mucous and serous membranes may be noted. There are enlargement and congestion of the liver and spleen, and frequently extramedullary hematopoiesis in the liver, spleen, lungs, kidneys, thyroid, thymus and lymph glands. Changes in the liver cells are reported as slight or not any in a few cases, while in most instances cloudy swelling and slight atrophy to extensive diffuse necrosis may be found, together with bile and iron pigment and "bile thrombi." Abnormal phagocytosis of the red blood cells by macrophages in the liver and spleen is demonstrable. Edema of the alveolar walls, leukocytic infiltration and small to fair-sized hemorrhages may be noted in the lungs. In a few cases yellow staining and necrosis of the ganglion cells in certain of the nuclear areas of the brain may be found.

While definite evidence of a hereditary transmission of this disease is lacking, its frequent familial occurrence is unquestionable. Healthy parents, normal pregnancies and short, easy deliveries are the rule, with normal healthy children frequently preceding the first affected child in birth.

The occurrence of universal edema of the fetus (fetal hydrops) or of idiopathic anemia of the new-born in siblings of the infant affected with icterus gravis neonatorum, together with suggestive similarities in the clinical and pathologic pictures, constitutes strong evidence for an identical etiology in these three disorders of the new-born.

Measured by criteria based on the foregoing observations concerning this disease, most of the existing theories of etiology are found insufficient in their explanations as to the probable or possible cause or causes.

The clinical symptoms suggest injury of the liver in those cases in which the disease is associated with erythroblastosis. Since an increased oxygen unsaturation of the arterial blood has been found associated with disease or injury of the liver, it seems probable that the erythroblastosis is secondary to anoxemia resulting from damage

to this organ, since diminution in hematopoietic activity is found to be coincident both with improvement in hepatic function in an affected infant and with increased oxygenation of the blood following birth in a normal child.

Considerable evidence permits the assumption of two fundamental pathologic processes as responsible for the symptoms and changes in this disease and in the related disorders usually associated with erythroblastosis: (1) abnormal destruction of erythrocytes and (2) hepatic dysfunction due to injury. In aregenerative congenital anemia abnormal destruction of erythrocytes by itself seems sufficient in most cases to explain the course of the disease. The primary influence giving rise to these pathologic processes in a series of offspring in the same family must be traced to the mother. The placenta seems to be the means of transmission of the destructive influence from mother to fetus.

An antigen-antibody reaction seems to explain best all aspects of these related disorders.

Obituaries

RICHARD HERMAN JAFFÉ, M.D.

1888–1937

Richard Herman Jaffé was born in Vienna forty-nine years ago (June 13, 1888), the son of a landowner whose estate was near Krakow, Poland. Shortly after his birth the family moved to Hamburg, but on the outbreak of the cholera epidemic in 1892 they returned to Vienna for permanent residency, and it was this environmental association that was the dominant influence throughout the years of his youthful development. At this time its cultural institutions, artistic, literary and educational; its beautiful environs, its historical associations, made Vienna, without doubt, Europe's most civilized capital. The University of Vienna, and particularly its medical faculty, not only had a glorious tradition but was then at the zenith of its clinical fame, attracting thousands of students from all over the world.

It was this atmosphere that naturally colored and molded Jaffé's point of view, his professional training and his attainment; it found expression in his tolerance, his subtle humor, his adaptability.

His first training was, of course, in the humanistic gymnasium; along with the fundamentals went equally valuable extracurricular training in music and the other arts; indeed, at this period his interest in music went so far that he seriously contemplated it as a field for his life work (he was a student of Reger), but finally he entered the medical school, from which he graduated in 1912. This was followed by study in various German clinics and laboratories and included some time spent with Abderhalden, at Halle, with Stoerck, and a year (1913-1914) at the Institute for Tropical Medicine, Hamburg. Returning to Vienna, he entered the laboratory of Paltauf (Sero-Therapeutic Institute), and it was probably Paltauf who had the greatest influence on his career during the following decade. In this institution, too, he came into close contact with a number of associates with whom he collaborated in scientific work. These included Pribram (now at Loyola University, Chicago) and Sternberg, as well as Silberstein.

The publications of this period included studies on the Unna-Pappenheim staining reaction in glands, studies on the Langhans cells of the pancreas and experimental observations on the Abderhalden reaction, on the histologic alterations in anaphylaxis and other phenomena.

With the outbreak of the World War he served with a number of divisional laboratories and for a short time was actually a captive of the

Russians when they entered Chernovitz. He managed to get back to the Austrian lines and was then appointed *Prosektor* at the first Garnissons Spital in Vienna.

Here an immense amount of autopsy material came under his observation and whole series of publications dealing with anatomic

RICHARD HERMAN JAFFÉ, M.D.
1888–1937

problems followed in the course of the next few years. Among them might be mentioned his studies of sarcoma of the gallbladder, myxoma of the heart, the histopathology of influenza, pathologic variation of the circumference of the aorta, ganglion neuroma of the adrenal, the anatomy of aviation death (traumatic rupture of the aorta) and the histopathology of typhoid changes of the liver. He summarized his observations in

1921 in a paper entitled "Pathology of War Experience." It is of interest to note that because of his careful pathologic work at the first Garnissons Spital the accuracy of clinical diagnosis increased progressively year after year until a very fine record of clinical diagnosis was finally established and maintained by the clinical staff.

In 1922 he was made a *Privatdozent* of the faculty of the medical school.

About this time Dr. Charles S. Bacon, professor of obstetrics at the University of Illinois and president of the medical staff of Grant Hospital, Chicago, visited Vienna. Grant Hospital had received as a gift (the Uhlein Fund) a sum sufficient to equip a modern hospital laboratory and to Dr. Bacon had been entrusted the task of seeking a well trained director for this new venture. While in Vienna his attention was directed to the work of Jaffé, and he induced Jaffé to leave Vienna and take the position at Grant Hospital.

On his arrival in Chicago, he was at once appointed to the staff of the department of pathology and bacteriology of the University of Illinois College of Medicine. With the opening of the Research and Educational Hospital (1924) the postmortem material was at once placed at Jaffé's disposal and, though limited in amount (approximately fifty postmortem examinations a year), the selection of the cases resulted in a series that offered much of interest.

It had long been obvious to his associates that Jaffé's ability could find proper expression only if he could have a large pathologic material at his disposal. Through the energetic intervention of Dr. Louis Schmidt, the chairman of the Board of Cook County Commissioners became interested and finally agreed to the inclusion of an adequate salary for a full time pathologist for the Cook County Hospital, with the understanding that Jaffé would fill the position.

In the decade that now followed, Jaffé built up an institution that became invaluable as a center of instruction not only for the staff of the Cook County Hospital but for a large group of practitioners, who found genuine inspiration in his Thursday conferences, as well as for a whole corps of loyal young assistants and associates, who crowded his laboratory to remain for a year or more to obtain instruction in pathologic technic.

Naturally his influence did not remain local but was carried far and wide by lectures in various cities, by his publications and by the work of his students, who soon came to occupy positions in the departments of pathology of various hospitals.

In the midst of all this activity Jaffé died suddenly on the night of Dec. 17, 1937. In the preceding six months he had been aware of a serious cardiovascular condition, which appeared to progress rapidly. With characteristic fortitude he continued his task. To his admonishing friends and advisers he merely replied that the work had to go on. And

the work did go on with the same indomitable steadfastness, with the same care and apparently with the same serene patience. To the very end he had time for every added task whether it involved the question of an intern, advice for a colleague or service on a special commission. He faced death with the same quiet, unobtrusive courage and with the same objectivity with which he approached the ordinary problems of the day.

For his colleagues his death was a great personal loss; for the whole medical profession of Chicago it signified the passing of a great clinical teacher of pathology.

WILLIAM F. PETERSEN.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Pathologic Anatomy

Myeloid Infiltrations in the Adrenals of Animals Bearing Certain Tumors. M. R. Lewis, Am. J. Cancer **30**: 95, 1937.

Areas of cell infiltration, showing some myeloblasts, one or more of them in mitosis, a number of myelocytes and many polymorphonuclear cells, were found in the adrenals of certain tumor-bearing animals. These myeloid infiltrations were not common to all tumor-bearing animals; they were limited to those bearing tumors the growth of which was accompanied by development of severe neutrophilia in the peripheral blood and myeloid hyperplasia of the spleen, bone marrow and certain lymph nodes. From the Author's Conclusion.

The Commonest Cause of Hypertrophy of the Right Ventricle—Left Ventricular Strain and Failure. W. P. Thompson and P. D. White, Am. Heart J. **12**:641, 1936.

Among 2,000 consecutive postmortem examinations, there were 704 cases of hypertrophy of the right ventricle to the extent that the wall measured 5 mm. or more in thickness. In about one fourth of these cases, no strain on either side of the heart was clearly evident. In 61 per cent of the remaining cases, the strain on the heart had been due to arterial hypertension, aortic valvular disease or infarcts of the left ventricle, and no factor producing primary right ventricular strain could be found. Left ventricular strain was, then, the commonest cause of hypertrophy of the right ventricle. Hypertrophy of the right ventricle was often found in cases with pure left-sided strain, regardless of the presence or absence of clear clinical evidence of failure of the left ventricle, but the presence of failure increased the degree of hypertrophy. The degree of hypertrophy of the right ventricle was almost as great in cases with pure left-sided strain as it was when there had been primary right-sided strain. A few examples of preponderant hypertrophy of the right ventricle were found in cases with pure left-sided strain in this series. In four of these electrocardiograms showed right axis deviation. Two other similar cases have been encountered, not in the series analyzed in this article. From the Authors' Summary.

Carbohydrate Metabolism, Local Acidosis and the Cytologic Picture in Inflammation. V. Menkin and C. R. Warner, Am. J. Path. **13**:25 (Jan.) 1937.

With the development of an acute inflammatory reaction, the carbon dioxide capacity of the cell-free exudate progressively diminishes. This is correlated with an increase in the hydrogen ion concentration and by a concomitant shift in the cellular composition from a polymorphonuclear to a mononuclear phagocytic phase. When the p_H drops below 6.7 or 6.5, most of the leukocytes appear to be injured, and frank suppuration ensues.

An inflammatory exudate manifests greater glycolytic activity than blood, as indicated by the higher level of lactic acid and the correspondingly lower concentration of sugar in the exudate.

Glycolysis increases as the inflammatory reaction progresses in intensity. Within several days, particularly if the reaction has been intensified by reinoculat-

ing the irritant, the concentration of lactic acid is considerably augmented and the result is localized lactic acidosis.

The evidences indicate that the mechanism of local acidosis in inflammation is therefore primarily referable to an increase in glycolysis and a consequent depletion of the alkali reserve. With the increase in the hydrogen ion concentration to a p_H below 7 polymorphonuclear leukocytes seem unable to survive, and the predominating infiltrating cell is the mononuclear phagocyte. Maintenance of an alkaline reaction resulting from a relative diminution in glycolytic activity is accompanied by a preponderance of polymorphonuclear leukocytes, with no subsequent shift in the cellular constituents of the exudate.

The available evidence indicates that the cytologic picture in an area of acute inflammation appears to be conditioned by the local p_H, which in turn depends on the rate of glycolysis and the depletion of the alkali reserve. The significance and implications of local disturbances in carbohydrate metabolism in determining the severity of an acute inflammation have been discussed. VALY MENKIN.

THE PRODUCTION OF BLOOD PLATELETS IN THE LUNGS. W. H. HOWELL and D. D. DONAHUE, J. Exper. Med. **65**:177, 1937.

A new fixing solution is described, which preserves the platelets and prevents contact hemolysis of the erythrocytes, so that counts of both corpuscles may be made in the same preparation. Comparative counts of platelets in arteries and veins show that arterial blood contains the larger number of platelets. This difference is accentuated under experimental conditions that cause a reduction in the number of platelets. It is concluded that new platelets are added to the blood in the capillary areas of the lungs, and that there is a corresponding destruction of platelets as the blood passes through the capillary areas of the systemic circulation. Perfusion of the lungs with a platelet-preserving solution, compared with that of other organs, gives evidence of the existence of a source of platelet material in the lungs. Histologic examination of the lungs with a technic adequate to give a differential staining of platelet material demonstrates the presence of giant cells in the lungs and supports the view that they are active in the production of platelets. In extra-uterine life giant cells are concentrated in the marrow and the lungs, with the maximum of their activity in platelet production in the lungs. FROM THE AUTHORS' SUMMARY.

LESIONS INITIATING RENAL CALCULUS. A. RANDALL, Surg., Gynec. & Obst. **64**:201, 1937.

The various theories as to the origin of the renal calculus give no information as to how, where and why such a body starts. Based on the postulates that an initiating lesion had to exist and that such a lesion should be sought for on or close to the renal papillae, Randall set out to test the validity of the theories. Roentgenographically most small calculi are found in the region of the renal papillae and in minor calices. Again, when small renal stones of short clinical duration are studied with a hand lens they prove always to present on one side a uniform crystalline surface while the opposite side shows a sunken facet with an encroachment and redundancy of the crystallization all about its edges, indicative of mural attachment. Examination of each minor calyx and its papilla by means of a hand lens in 104 pairs of kidneys obtained at autopsy revealed lesions believed to be the starting points of calculi. The initial lesion occurs on the papilla, generally on the lateral wall or near its tip, ranges in size from that of a minute dot to a diameter of 2 or 3 mm., has a sharp or stellate outline and for want of a better term is called a "milk patch." Sections disclose a deposit of calcium unaccompanied by any inflammation in the wall of the renal papilla and below the surface epithelium, which for a time remains intact but later breaks down, exposing the calcium and allowing the secondary deposition of salts from the urine. When the concretion becomes detached, the mural calcium plaque is broken off with it. The secondary salts deposited on the primary patch are calcium phosphate and calcium oxalate. WARREN C. HUNTER.

CALCIUM NEPHROSIS IN PYLORIC OR DUODENAL STENOSIS. E. PÉREZ-CASTRO, Beitr. z. path. Anat. u. z. allg. Path. **99**:107, 1937.

In a series of fifty cases of pyloric or duodenal stenosis most frequently due to chronic ulcer and carcinoma and accompanied by vomiting with reduction of the blood chlorides, there were five cases in which the cortical tubules of the kidney after silver impregnation (von Kossa) presented more or less marked accumulations of calcareous spherical masses. These were associated with loss, flattening and regeneration of the tubular epithelium.

R. J. LEBOWICH.

STRUCTURAL CHANGES OF MECHANICAL ORIGIN IN ORGANS REMOVED DURING OPERATION OR NECROPSY. L. ASCHOFF, Med. Klin. **33**:149, 1937.

According to Aschoff, the autolytic and putrefactive changes normally occurring in operatively removed organs and those examined post mortem may be altered through rough mechanical injury. Hemorrhage occurring in all operatively removed organs, such as the appendix, is to be regarded as of possible traumatic origin. Clumping and fusion of nuclei observed in lymphocyte-rich organs, such as the tonsil, appendix, stomach and intestine, are due to contusion. Clumping is also seen in the epithelial tissue of the thyroid gland, giving a resemblance to fetal adenoma. Diagnostic error may result from desquamation of the epithelium in the serous cavities, lower respiratory tract, gastro-intestinal tract and urinary tract and from postmortem dissolution of the endothelium of the blood vessels, of the hepatic parenchyma and of the follicular epithelium of the thyroid gland. Fragmentation of the myocardium is a postmorten phenomenon and is not to be confused with cement lines.

FREDERICK STENN.

HISTOLOGIC DIAGNOSIS OF MEASLES. C. WEGELIN, Schweiz. med. Wchnschr. **67**:1, 1937.

The Warthin-Finkeldey giant cells were found in the germ centers, neighboring lymphatic tissue and tunica propria of the mucosa of the vermiform appendix removed surgically from each of three patients during the prodromal stage of measles. The giant cells were round or oval, often measured over 100 microns in diameter and contained from 50 to 100 chromatin-rich nuclei, which frequently were found undergoing pyknosis and karyorrhexis. Ingested lymphocytes and nuclear fragments were present in the protoplasm of these cells. In one case the mesenteric lymph nodes showed a primary tuberculous infection with central caseation and calcification, surrounded with large epithelioid cells and Langhans giant cells, while the rest of the lymph nodes revealed giant cells similar to those seen in the appendix. The Warthin-Finkeldey giant cells indicate a reaction to infection by the virus of measles. The frequent localization in the appendix suggests that this organ may be a portal of entry for the virus, which then spreads to the mesenteric lymph glands.

FREDERICK STENN.

ANNULAR HEMORRHAGES OF THE BRAIN. K. WOLFF, Virchows Arch. f. path. Anat. **298**:98, 1936.

Wolff recognizes three types of hemorrhage into the brain as the result of trauma:

1. More or less massive hemorrhage into the traumatized and lacerated brain tissue. Such a distintegrative hemorrhage occurs most often in the white matter.

2. Hemorrhage into the perivascular space of His. A hemorrhage of this type follows the course of the involved vessel for a relatively long distance.

3. Annular hemorrhage. This occurs in a restricted area about a small vessel at some distance from the main hemorrhage; it appears circular in cross-section but is actually spherical.

It is with a detailed study of the annular hemorrhage that the author's lengthy contribution deals. Hemorrhages of this type occur not only in the brain tissue surrounding larger traumatic hemorrhages but are seen also in a variety of infections and intoxications. Their presence about apoplectic hemorrhages has led to the contention that they are indicative of a primary disturbance of the cerebral circulation, to which the massive apoplexy is secondary. Wolff has selected annular hemorrhages due to trauma for study because the time relationship between the causative factor, the trauma, and the hemorrhages could be more accurately determined. The ring hemorrhages of traumatic origin are compared with those occurring in infection and intoxication. Morphologically the annular hemorrhage is characterized by its restricted localization in both the transverse and the longitudinal axis of the vessel. At the center is the small vessel filled with blood; about this is a narrow clear zone, and peripheral to this is the circumscribed hemorrhage into the surrounding brain tissue. From his study Wolff concludes that annular hemorrhages are not the result of a primary disturbance of the cerebral circulation. They result from local damage to the smallest vessels by trauma or toxic substances. The vascular injury leads to necrosis of the wall of the vessel and to injury of the barrier between the blood and the tissue (*Dysoria*). The contents of the vessel, the wall of the vessel and the immediately surrounding tissue are to be looked on as a unit in their reaction to the injury. The occurrence of identically similar hemorrhages in certain infectious diseases leads Wolff to postulate that such hemorrhages constitute a reactive phenomenon the purpose of which is to blockade the injured vessels.

O. T. SCHULTZ.

CHANGES IN THE LEFT AURICULAR ENDOCARDIUM IN MITRAL INSUFFICIENCY. R. BÖHMIG, Virchows Arch. f. path. Anat. **298**:161, 1936.

In 129 of 200 consecutive unselected necropsies there were observed fine ripple-like ridges of the endocardium of the left auricle at the base of the mitral segments. They vary in number and run in various directions. Microscopic examination revealed that they are not the result of inflammatory reaction. Careful measurements showed the mitral orifice to be somewhat enlarged and the mitral segments somewhat shortened. They are held to be the result of mechanical factors operative in clinically unrecognizable slight grades of mitral insufficiency.

O. T. SCHULTZ.

HISTOLOGIC DIFFERENTIATION BETWEEN MYELOID AND LYMPHATIC LEUKEMIA IN THE LIVER. C. CORONINI, Zentralbl. f. allg. Path. u. path. Anat. **67**:81, 1937.

Myeloid leukemic alterations in the liver usually are characterized by an increase in the number of white cells in the blood stream and by intracapillary myeloid cell clumps within the lobules and periportal fields. The myelopoiesis is usually accompanied by erythropoiesis, so that these cell masses resemble hematopoietic centers. Lymphatic leukemic forms are characterized by proliferation of the endothelium of lymph vessels, and these cell masses occur especially in the periportal field and in the adventitia of the veins of the hepatic circuit.

GEORGE J. RUKSTINAT.

UNUSUAL EMBOLI FOLLOWING RUPTURE OF AN AORTIC ANEURYSM INTO THE ESOPHAGUS. G. ROMHÁNYI, Zentralbl. f. allg. Path. u. path. Anat. **67**:273, 1937.

In the body of a man 39 years old who died suddenly of an aortic aneurysm which ruptured into the esophagus, grape skins were found in the right common carotid artery, right middle cerebral artery and common iliac arteries. A grape seed was found in the superior mesenteric artery. The man had eaten the grapes shortly before death and apparently had an incomplete emesis just before rupture of the aneurysm. There was blood in the stomach but none in the esophagus.

GEORGE J. RUKSTINAT.

Pathologic Chemistry and Physics

DISTRIBUTION OF LIPIDS AND MINERALS IN SERUM AND ERYTHROCYTES IN PERNICIOUS ANEMIA. H. H. WILLIAMS and others, J. Biol. Chem. **118**: 599, 1937.

Coordinated hematologic, chemical and physicochemical examinations were made of the blood in pernicious anemia. The serum minerals were unaffected, but the plasma lipids showed an increase of neutral fat with a decrease of cholesterol esters and phospholipid. These return to normal levels after treatment. In relapse, the red cells contained an excess of cholesterol esters and a deficiency of free cholesterol and of phospholipid. Both the cation and anion content were increased, owing chiefly, in the case of the cation, to an increase in potassium and, in the case of the anion, to an increase in the hemoglobin. The chemical composition of the erythrocytes returned to normal with improvement. R. J. LEBOWICH.

TENSION AT THE SURFACE OF MACROPHAGES. H. SHAPIRO and E. N. HARVEY, J. Cell. & Comp. Physiol. **8**: 21, 1936.

The appearance of macrophages laden with oil globules was induced by the injection of mineral oil into the peritoneal cavity of the rabbit. It is calculated that the tension at the surface of the macrophages has an average maximal value at 23-28 C. of about 2 dynes per square centimeter. This is arrived at by the determination of the centrifugal force necessary to pull globules of known size through the surface. R. J. LEBOWICH.

BIOLOGIC STUDIES OF EXTREME PRESSURES. J. BASSET, M. MACHEBOEUF and E. WOLLMANN, Ann. Inst. Pasteur **58**: 58, 1937.

Cells of higher animals and neoplastic cells were killed by a pressure of about 1,800 atmospheres. The ultramicroscopic viruses were inactivated at a pressure under 5,000 atmospheres, except the virus of Borna disease, which was destroyed at 6,500 atmospheres. Nonsporulating bacteria survived 5,000 but not 6,000 atmospheres. Some proteins studied were not altered, but some were irreversibly coagulated, at pressures over 7,000. Enzymes and bacterial toxins could not be inactivated under 19,000 atmospheres regularly. Spores survived even 20,000 atmospheres regularly. The bacteriophage reacted more like a virus than like an enzyme in this respect. The sarcoma of Rous and the papilloma of the rabbit survived, respectively, 2,000 and 4,000 atmospheres, but other transmissible neoplasms did not; the former would thus fall with viruses and the latter would not, with respect to this phenomenon. Serologic observations indicated that antigenic specificity was destroyed by pressure. It appeared, however, that the shock-producing power of serum was destroyed by pressures which did not destroy antitoxin, an observation which might prove helpful. MAX MARSHALL.

ION CONCENTRATION OF ALLERGIC-HYPERERGIC INFLAMMATION. R. KNEPPER, Klin. Wchnschr. **16**: 188, 1937.

The tissues in normergic inflammation have an acid reaction, the p_H ranging between 7.4 and 6.8 (Schade and others). According to Knepper, in allergic-hyperergic inflammation, such as the Arthus phenomenon, the p_H indicates a distinctly alkaline reaction, varying between 6.95 and 8.15.

FREDERICK STENN.

Microbiology and Parasitology

CULTIVATION OF THE VIRUS OF ST. LOUIS ENCEPHALITIS. R. W. HARRISON and E. MOORE, Am. J. Path. **13**:361, 1937.

Three strains of the virus of St. Louis encephalitis have been successfully cultivated in vitro in the presence of living cells. The mediums employed were

minced mouse or chick embryo tissue suspended in Tyrode's solution either with or without rabbit serum. Mediums containing normal serum supported the growth of virus better than mediums without serum. When rabbit serum possessing neutralizing antibodies against the virus was employed in cultures, the infectious agent was not recovered. Two strains of the virus were propagated in serial passage in developing chicks by inoculation on the chorio-allantoic membrane. The virus was recovered from the brain, liver and spleen, as well as from the chorio-allantoic membrane, of each chick embryo used for passage. Histologic changes occurred in all three germ layers of the embryonic membrane. Young chicks are to some extent susceptible to the virus.

From Authors' Summary.

Experimental Infection of Some Reptiles, Amphibia and Fish with Serratia Anolium. H. J. Clausen and F. Duran-Reynals, Am. J. Path. **13**:441, 1937.

Serratia anolium, a bacterium which induces tumor-like lesions in the iguanid lizard Anolis equestris, was injected into other cold-blooded animals, as well as into warm-blooded animals of several species. In all the forms of reptiles or amphibians used the lesions were produced and appeared to be similar in all respects to those of the natural disease. The causative bacterium was easily isolated and cultured from the lesion as well as from the heart blood. Injection of the bacterium into cold-blooded forms of the same species of experimental animals but kept at room temperature (37 C.) always produced general pathogenic effects and proved fatal in most instances. In the warm-blooded animals used, the bacterium was nonvirulent, and no pathogenic effects were noted in any case.

From Authors' Summary.

Oral Tuberculous Lesions. C. C. Darlington and I. Salman, Am. Rev. Tuberc. **35**:147, 1937.

Twenty-four cases are grouped as follows: class 1, twelve cases in which the apex and socket were involved; class 2, ten cases in which the oral mucous membrane was involved; class 3, one case of tuberculous osteomyelitis, and class 4, one case of tuberculous lymphadenitis. It is concluded that tuberculous lesions of the mouth may have a dental application. In the cases in which tuberculosis attacks the apex or the socket (especially the former) there most frequently is an accompanying destruction of bone, apparent roentgenographically, around the tooth root. Tuberculosis of the mucous membrane most frequently involves the gingiva, which presents a mouse-eaten, ulcerative appearance, usually associated with looseness of the tooth. Both occur secondarily to advanced pulmonary tuberculosis.

H. J. Corper.

Benign Lymphocytic Meningitis (Aseptic Meningitis). C. M. Dummer, R. A. Lyon and F. E. Stevenson, J. A. M. A. **108**:633, 1937.

Symptoms of benign lymphocytic meningitis were observed in a group of twenty-two children during the year 1935. The peak of this small epidemic occurred in the months of July and August. The disease developed in widely separated areas of the community except in two instances, so that contact with known patients could not have been responsible for its spread. Characteristic symptoms of the disease were headache, vomiting and abdominal pain. On physical examination rigidity of the neck and positive Kernig and Brudzinski signs were most frequent. Most of the cerebrospinal fluids contained from 50 to 200 cells, chiefly lymphocytes, and the globulin content was greater than normal in about half of the group. The fever was of short duration, and in nineteen of the twenty-two cases the leukocytes of the blood were not increased above normal figures.

Recovery was rapid and complete. More than half of the group were examined from six to eight months after recovery from the disease but no evidence of residual symptoms and signs or of changes in behavior could be discovered. The mildness of the infection, its epidemic characteristics and the absence of any other disease in the patients or in the community to account for the illness led the authors to suspect that it was a definite disease entity, caused possibly by a virus.

From Authors' Summary.

Significance of Serologic Types Among Meningococci. S. E. Branham, J. A. M. A. **108**:692, 1937.

Classifications of meningococci that were worked out in the years 1909-1918 represented true serologic relationships which can be plainly recognized today.

Certain changes in these relationships have taken place: Types I and III have become so closely interrelated that separation into two types no longer seems to be of definite value in practical everyday work. This I-III, or A, group has become markedly predominant in nearly all parts of the world that have been heard from.

On the other hand, types II and IV have, in the United States at least, become entirely distinct from each other, so that they represent two separate groups. There seem to be three types of meningococci: I-III, II and IV. The designation B cannot be well applied to a combination of two such distinct groups as II and IV.

Studies made long ago and at the present time seem to indicate a greater number of type II strains in carriers. This large number of carriers of strain II in proportion to the very small number of type II cases brings up this question: Is II less pathogenic than I-III? This idea finds some support in the following fact: Although 70 per cent of all strains isolated from blood were type II, nearly all the cases of meningococcic endocarditis in which the type has been determined have been due to the I-III group. There is some evidence that type II is especially apt to be responsible for septicemic and generalized forms of meningococcic infection, which may be relatively mild or chronic.

Both the endotoxins of Gordon and the soluble toxins reported by Ferry are produced to a greater extent by the I-III group. Thus one finds at present a predominance of that group of meningococci which seems to be both more invasive and more toxic.

From Author's Summary.

Rapid Invasion of the Body Through the Olfactory Mucosa. G. Rake, J. Exper. Med. **65**:303, 1937.

Prussian blue particles pass rapidly from the surface of the olfactory mucosa and within two minutes are found in the tissue spaces, in the blood and lymph vessels, in the spaces about the olfactory nerve fibers and in the subarachnoid space and pia-arachnoid membrane. There is great affinity of the pigment particles for the olfactory sensory cells. Preliminary treatment of the olfactory mucosa with tannic acid does not alter the speed with which this absorption occurs. It does, however, cause inflammation of the mucosa and appears to prevent the pigment from entering the olfactory sensory cells. Both pneumococci and Salmonella enteritidis pass through the olfactory mucosa and reach the tissue spaces, vessels and subarachnoid space with the same rapidity as the pigment. This can be demonstrated both microscopically and by distribution tests. They invade by passage between the cells of the mucosa, and there is no apparent affinity of the organisms for the olfactory sensory cells. Tannic acid treatment of the olfactory mucosa in no way alters this invasion of organisms through the mucosa. The pantropic virus of equine encephalomyelitis was detected in the forebrain as promptly as were pigment and bacteria; neurotropic viruses, however—those of encephalitis B (St. Louis), rabies and louping ill—were not demonstrated in less than twenty-four hours.

From the Author's Summary.

REINFECTION (SECOND ATTACK) IN EXPERIMENTAL POLIOMYELITIS. S. FLEXNER, J. Exper. Med. **65**:497, 1937.

Monkeys which have recovered from an attack of experimental poliomyelitis are subject to reinfection by the nasal route. Second attacks of the disease result from inoculation with the specimen of virus used to produce the first attack and with specimens of different origin. Reinfection takes place in monkeys which have recovered from mild and from severe attacks and in convalescent animals which have been subjected to hyperimmunization. The two year quiet period proposed by Still to separate relapses from second attacks, judged from the monkey, is probably excessive. Until greater attention is given the reinfections of varying intensities in man, conclusions on this point must be wholly tentative.

FROM AUTHOR'S SUMMARY.

VIRUS OF POLIOMYELITIS: AN IMMUNOLOGIC COMPARISON OF SIX STRAINS. J. D. TRASK and others, J. Exper. Med. **65**:687, 1937.

Qualitative immunologic differences exist between early passage strains or so-called human strains of the virus of poliomyelitis. These differences show a relationship to the epidemic source of the virus and are exemplified in this study by four strains isolated in different years during an eastern and western epidemic of poliomyelitis.

FROM AUTHORS' CONCLUSIONS.

SPONTANEOUS ENCEPHALOMYELITIS OF MICE, A NEW VIRUS DISEASE. M. THEILER, J. Exper. Med. **65**:705, 1937.

The characteristics of a filtrable virus obtained from mice found spontaneously paralyzed and showing lesions of encephalomyelitis are described.

FROM AUTHOR'S SUMMARY.

INFECTION OF MICE WITH MUCIN SUSPENSIONS OF HAEMOPHILUS INFLUENZAE. L. D. FOTHERGILL, J. H. DINGLE and C. A. CHANDLER, J. Exper. Med. **65**:721, 1937.

It has been shown that the ability of virulent Haemophilus influenzae to kill mice is enhanced by suspending the organism in mucin. The occurrence of a true infection, characterized by in vivo multiplication of the organism, has been established. The necessity for using a pure inbred strain of mice for this type of study has been confirmed. The increase in virulence of a strain of H. influenzae in mucin by repeated passage through mice has been shown. The usefulness of this method in studies of passive immunization has been demonstrated.

FROM AUTHORS' SUMMARY.

THE PROBABLE NATURE OF THE INFECTIOUS AGENT OF TRACHOMA. L. A. JULIANELLE, R. W. HARRISON and M. C. MORRIS, J. Exper. Med. **65**:735, 1937.

The infectious agent of trachoma can be freed from extraneous bacteria by passage through the rabbit testicle. The infectious agent multiplies little, if at all, during such passage but in many instances retains its infectivity undiminished. No specific changes occur in the rabbit testicle incidentally to the passage. On rare occasions the agent of trachoma may be freed from bacteria by intracerebral passage. The brain tissues show no specific reaction. Experiments with Seitz, Kramer, Berkefeld and Elford filters confirm the general observation that the infectious agent is filtered with difficulty. Culture of tissues containing the infectious agent (conjunctiva, rabbit testicle, brain, etc.) under a wide variety of conditions proved unsuccessful in the cultivation of the agent. The agent is inactivated by bile, silver nitrate, phenol, cocaine, antimony and potassium tartrate, and gentian

violet. It is inactivated at a temperature between 45 and 50 C. in fifteen minutes. Attempts to preserve the infectious agent in glycerin were unsuccessful. The accumulated evidence suggests that the infectious agent of trachoma is a virus.

FROM AUTHORS' SUMMARY.

SURVIVAL OF OXYGEN AND WATER DEPRIVAL BY TUBERCLE BACILLI. T. S. POTTER, J. Infect. Dis. **60**:88, 1937.

The severest deprival of oxygen and of water yet accomplished does not guarantee the death of tubercle bacilli. Under such conditions the bacilli in substantial numbers retain their viability. In fact, avian bacilli subjected to such conditions at body temperature for more than a year consistently survive in substantial numbers; at room temperature they survive for at least two years.

THE CARBOHYDRATE GRADIENT OF CERTAIN MICRO-ORGANISMS. A. G. WEDUM and B. L. GOLDEN, J. Infect. Dis. **60**:94, 1937.

A survey was made of the fermentation of dextrose, mannose and fructose by some 800 cultures of the American Type Culture Collection. Analyses for reduction of sugar were made as a supplement to the use of acid indicators. The findings emphasize anew the inadequacy of the acid indicator system in determining utilization of carbohydrate by many types of micro-organisms.

The primary purpose of the survey was to find organisms which might serve as chemical reagents, particularly in studying interconversion of hexoses in mildly alkaline solutions. A number of organisms were found to utilize dextrose and fructose but not mannose. Pasteurella boviseptica 4327 utilized dextrose readily, a small amount of fructose only after long incubation and mannose not at all. Staphylococcus aureus 427 utilized dextrose readily and mannose and fructose only after long incubation. Attention is called to Leptothrix 4804, which is remarkable in its utilization of fructose in the absence of ability to ferment dextrose and mannose. Leptothrix 4804 is an outstanding exception to the theory of the carbohydrate gradient which names dextrose as the most easily utilizable carbohydrate.

FROM THE AUTHORS' SUMMARY.

LACK OF FITNESS AS THE PREDISPOSING FACTOR IN PNEUMONIA AND IN THE COMMON COLD. A. LOCKE, J. Infect. Dis. **60**:106, 1937.

Infections of the type encountered in pneumonia and in the common cold may result from slowing up of the mechanisms normally continuously employed in the removal of invading bacteria from the circulating blood. The slowing up may be body wide. It is manifested in the rabbit in a lengthening of the time required for recovery of a normal temperature after chilling and in man in a lengthening of the time required for replacement of oxygen after exercise.

The speed with which the temperature can be recovered in the rabbit and the speed with which oxygen can be replaced in man under defined conditions have been used to establish a rating of fitness, or an index of ability to support rapid performance of work. Ninety-two per cent of twelve rabbits with a rating of more than six tenths of the possible maximum survived intravenous infection with small numbers of virulent type I pneumococci without intermediate fever and without apparent injury. One hundred per cent of eleven rabbits with a rating of less than five tenths of the possible maximum died within three days after such infection. Sixty-nine per cent of sixteen rabbits with the higher ratings gave negative blood cultures for more than twenty-six hours after the establishment of an intradermal focus of pneumococcic invasion. Forty-four per cent were carried through to recovery. Not one of sixteen rabbits with the lower ratings were able to check invasion following intradermal infection or to survive.

Sixty-four per cent of a mixed group of twenty-eight persons with ratings above six tenths of the maximum reported one cold or less between Oct. 1, 1935,

and May 1, 1936. Eighty per cent of an equivalent group of fifteen persons with ratings below five tenths reported four colds or more for the same period.

Influences which tended to lower or improve the rating in the rabbit were followed by parallel decreases in ability to survive pneumococcic infection. Among the factors which impaired fitness were: maintenance in overheated quarters, morphine poisoning, toxemia, and starvation when this was accompanied by weight losses in excess of 2.5 per cent per day. Among the factors which improved fitness were: removal to markedly cooler quarters, subcutaneous injections of from 0.1 to 0.4 cc. of anterior pituitary extract, intravenous injections of 1 cc. of an extract of adrenal cortex, intravenous injections of 2 cc. of a 20 per cent solution of an extract of liver, and feedings of the same extract in consecutive daily doses of 20 cc.

FROM AUTHOR'S SUMMARY.

FURTHER ADVANCES IN THE STUDY OF MICROBIC DISSOCIATION. P. HADLEY, J. Infect. Dis. **60**:129, 1937.

"The foregoing account of the many investigations presented in this review relating to dissociative variation has a particulate significance for many special problems. But it offers, at the same time, the vantage point for a broader outlook upon the nature and scope of what is commonly termed 'the science of bacteriology.'

"This science, compared with some others such as chemistry and physics, for example, manifests several peculiarities, clearly recognized by those who have felt that, despite its important contributions to human welfare, there has 'been something wrong about bacteriology' as a science. It appears possible to define some of these peculiar characteristics. First, there is probably no other science in which the 'pure' scientific aspects manifest so small and undeveloped a structure in proportion to its valuable applications. Second, it is probable that in no science so much as in this have there been performed so many experiments and investigations the net results of which have been of such slight importance to the mother science. Third, there is probably no science in which so large a proportion of the workers have so small an interest in the fundamental problems of the pure science. This strange circumstance, apparent to all who give serious consideration to the broader problems of bacteriology, must have an explanation, and I believe it may be found in the following facts.

"In the first place, pure bacteriology must be regarded as a still relatively undeveloped subject. This is due in part to restrictions placed upon its progress by the demands of the monomorphic conception many years ago. It is also due to a considerable extent to the circumstance that bacteriology has, since the early days of the morphologists, been submerged in a maze of attempted applications. This has resulted in side-tracking investigators from the less profitable, and considerably more dull, problems of consequence to the pure science. In this connection, one may agree with Topley and Wilson when they write: 'It is almost true to say that the present position of bacteriology is due to the fact that there have been no bacteriologists. From Pasteur onward, bacteriologists have been more interested in what bacteria do than in what they are; and much more interested in the ways in which they interfere with man's health and pursuits than in the ways in which they function as living beings.'

"In the second place, there has existed, in the rather small parcel of knowledge represented by the subject-content of bacteriology, no integrating principle. Bacteriology has had no 'laws' resembling in any way those established long ago for other sciences. When one speaks of the 'law' or 'principles' of bacteriology, close examination shows that these laws are the laws of physics and of chemistry, or of physiology. It is probably for this reason that so large a proportion of contributions to bacteriology have nothing to tie to; no background of enduring fabric in which the observations can be integrated, either with the effect of supporting a principle already established, or of creating a new one. It is also because of this situation that some have even been inclined to question whether bacteriology is entitled to the rank of a science.

"Some years ago, James Truslow Adams in his entertaining biography of the Adams family, remarked: 'I see no use and much disadvantage in calling anything a science which has not so succeeded in arranging its facts and observations as to have disclosed 'laws,'—that is, uniform modes of behavior which show regular recurrence and thus possess predictability.' While many may not accept this criterion of what is entitled to rank as a science, the quotation given above reflects with considerable faithfulness a situation that has existed in bacteriology since its beginnings. Indeed, bacteriology has 'no laws,' and almost 'no bacteriologists.' These lacks, which so frequently seem to be accepted as a matter of course, measure the handicaps under which this science has labored and developed.

"But what is the bearing of all this on the newer studies on dissociative variation? In the first place it is apparent that, not since earlier days when morphological studies were paramount, have so many bacteriologists applied themselves to problems of pure bacteriology as during the past decade; for these workers have in considerable number attempted to expand the knowledge of microbic dissociation; and the most fundamental problems in this field are problems in pure bacteriology. Second, it is unquestionably true that a greater body of facts relating to the nature, organization and behavior of bacteria has been acquired during the past fifteen years than had been gained during the previous half century of endeavor under the older viewpoint. Finally, the most important, it appears that the results obtained in studying the biology of bacteria from the new viewpoint, freed from all the inhibiting influences of the monomorphic conception, have made possible, for the first time in the history of bacteriology, the development of certain integrating principles that soon may be introduced into the science. In other words, it is apparent that there are beginning to evolve some of those important facts upon which the first definite 'laws of bacteriology' may be based; and from the firm establishment of which bacteriology may sometime emerge to take its place as an actual, rather than as an implied science."

FROM AUTHOR'S CONCLUSIONS.

EXPERIMENTAL INFECTION WITH HAEMOPHILUS PERTUSSIS PRODUCED IN THE MOUSE BY INTRANASAL INOCULATION. F. M. BURNET and C. TIMMINS, Brit. J. Exper. Path. **18**:83, 1937.

Intranasal administration of cultures of Haemophilus pertussis to anesthetized mice resulted in characteristic pneumonic lesions, and large doses were rapidly fatal. Histologic sections showed interstitial pneumonia and typical intense proliferation of bacilli in the mucus lying on the ciliated surface of the bronchial epithelium. A significant degree of immunity could be demonstrated after intraperitoneal inoculation of living cultures or of formaldehydized vaccines. The method may be of value in determining the antigenic efficiency of vaccines prepared for the prophylaxis of whooping cough in children.

FROM AUTHORS' SUMMARY.

THE INVASIVENESS OF A STRAIN OF STREPTOCOCCUS HAEMOLYTICUS. M. G. PRADHAN, Brit. J. Exper. Path. **18**:90, 1937.

It has been clearly demonstrated by these experiments that a spreading factor is elaborated by the hemolytic streptococcus in the presence of serum during the early period of growth. That this substance is distinct from hemolysis was shown by demonstrating that the sixteen hour filtrate, having perhaps a slightly better hemolytic potency than the two hour filtrate, did not possess any spreading factor. As growth proceeds, this factor is either destroyed or neutralized, possibly by products of the metabolism of the cocci. It is elaborated only in mediums containing a high proportion of serum. The necessity of serum suggests that it can be produced in even greater proportions in the animal body, where encapsulation of the cocci in the early stages of multiplication has also been found to occur. As mentioned before, Day has suggested that his V antigen is intimately attached

to the surface of the organisms and is also free in the medium. It seems to follow from the present experiments that Day's V antigen is probably identical with the capsular substance of the young cocci and that when set free in the surrounding medium it constitutes "the spreading factor." Young cultures were found to be inagglutinable by an immune serum high in agglutinin which was prepared against the usual eighteen hour culture, and it seems reasonable to suppose that the young capsulated cocci are similarly resistant to the defense mechanism of the host. The role of the spreading factor in relation to infectivity is further brought out in the experiments showing the enhancing action which this factor exerts on the power of an avirulent strain to produce an inflammatory lesion in the rabbit's skin. The factor responsible for the invasive power of this streptococcus seems therefore to be the capsular material evolved by the cocci only in the early stages of multiplication. Surrounding the cocci as a formed capsule this substance may enhance their resistance to phagocytosis; free in the surrounding medium, it favors their spread through the host's tissues.

FROM AUTHOR'S SUMMARY.

MIXED INFECTIONS WITH HUMAN AND BOVINE TUBERCLE BACILLI IN MAN. A. S. GRIFFITH, Tubercle **18**:193, 1937.

Although man is exposed to tuberculous infection from bovine as well as from human sources, it is rare to find more than one type of tubercle bacillus in human tuberculosis. This has been the experience in Germany (8 in 1,027 cases up to 1932), Denmark (6 in 3,000 cases) and Great Britain (10 in 6,000 or more cases), where the most extensive investigations of the types of tubercle bacilli in human tuberculous lesions have been made. Griffith reports a case of infection with human and bovine bacilli in a young woman 18 years old who died of tuberculous meningitis. Tubercle bacilli of the bovine type alone were obtained from the meninges, while the lung yielded both human and bovine bacilli. It is believed that the human bacillus was the secondary invader.

H. J. CORPER.

Immunology

PROTECTIVE EFFECT OF IMMUNE PLASMA AGAINST THE DELETERIOUS INFLUENCE OF STREPTOCOCCAL EXTRACT ON HYPERSENSITIVE CELLS. J. K. MOEN, J. Exper. Med. **65**:587, 1937.

Plasma from guinea pigs that were chronically infected with hemolytic streptococci of group C, neutralized the components of bacterial extract which exert marked toxic action on hypersensitive cells in vitro. The neutralizing capacity of these immune plasmas is relatively specific for the bacterial extract; it is not due to a variable nonspecific effect on normal or hypersensitive tissue cells. A rough correlation between the relative neutralizing capacity and the agglutinin titer of immune plasma suggests that the former may be a manifestation of antibody action. The neutralizing capacity of the plasmas of guinea pigs explains, at least in part, their tolerance of chronic hemolytic streptococcic lymphadenitis, since such soluble bacterial products as may be absorbed from foci of infection would probably be neutralized before they could exert a deleterious influence on the hypersensitive cells of the animals.

FROM AUTHOR'S SUMMARY.

SEROLOGIC TYPING OF STREPTOCOCCUS HAEMOLYTICUS. R. H. PAULI and A. F. COBURN, J. Exper. Med. **65**:595, 1937.

The cross-reactions which interfere with serologic identification of hemolytic streptococci are due to anticarbohydrate in the serum used for typing. This antibody can be removed easily by absorption with purified streptococcus carbohydrate, and identification of type is then readily established. The serologic classification

of hemolytic streptococci from throat infections contracted in New York during 1935 and 1936 showed predominance of types 4, 13 and 22. Type 13 appeared to be the most serious in initiating rheumatic activity during this period of observation.

FROM AUTHORS' SUMMARY.

ELECTROPHORESIS OF PURIFIED ANTIBODY PREPARATIONS. A. TISELIUS, J. Exper. Med. **65**:641, 1937.

Electrophoretic mobilities of antibody preparations isolated from type-specific antipneumococcus horse and rabbit serums, measured over a range of p_H values, show that these preparations are distinctly different from normal serum proteins in their electrochemical properties.

FROM AUTHOR'S SUMMARY.

A QUANTITATIVE THEORY OF THE PRECIPITIN REACTION. M. HEIDELBERGER and F. E. KENDALL, J. Exper. Med. **65**:647, 1937.

The reaction between the specific polysaccharide of type III pneumococcus and homologous antibody in rabbit serum is quantitatively accounted for by expressions similar to those derived from the mass law for the corresponding horse serum. Preliminary data are also given for the type I reaction. Differences and similarities of the reaction with antibodies produced by the two animals are discussed. Calculations are made of the equivalent composition of the specific precipitate at various reference points in the reaction range. Certain theoretical and practical implications of the findings are pointed out.

FROM AUTHORS' SUMMARY.

RELATIONSHIP BETWEEN NASAL AND HUMORAL ANTIPOLIOMYELITIC SUBSTANCES. B. F. HOWITT, J. Infect. Dis. **60**:113, 1937.

The in vitro neutralization test was positive on thirty-six (59 per cent) of the serums and fourteen (22.9 per cent) of the filtered nasal washings from sixty-one persons examined. No particular correlation could be discerned between the presence of serum that reacted positively and the presence of nasal filtrates that reacted positively, although the latter occurred in more so-called normal adults than recovered poliomyelitic persons. In nine cases both serum and nasal filtrate gave positive results; in five the washings were antiviral but the serum was nonreactive, while in 27 the serum gave a positive reaction and the filtrate did not react.

About the same number of reactive filtrates were found among those with abnormal nasal conditions as among those with normal.

Of serums of normal adults, 71.3 per cent were antiviral, as compared with 60 per cent of the serums from those recovered from poliomyelitis while, of serums from normal children, 55 per cent were antiviral, as compared with 40 per cent of those from the group recovered from poliomyelitis.

Concentration of nasal filtrates by the Flosdorf and Mudd lyophil apparatus was of value in securing more positive neutralization tests.

No antiviral substances were found in the nasal washings of five immunized monkeys taken at different stages of immunity. Their tissue immunity failed to run parallel with their humoral response, since three animals had neutralizing antibodies in their serum without showing resistance to virus intracerebrally inoculated.

FROM THE AUTHOR'S SUMMARY.

BOTULINUS ANTIGENS. L. S. MCCLUNG, J. Infect. Dis. **60**:122, 1937.

A study has been made of the heat-labile and heat-stable antigens of an extensive collection of strains of Clostridium botulinum, Clostridium parabotulinum and other related species. It was found that the subgroups based on the agglutination reactions of the cultures of Cl. parabotulinum are dependent on the heat-labile or flagellar antigen. Strains deficient in but not devoid of the heat-labile antigen

were believed to be present in the collection. That all strains of Cl. parabotulinum possess the same heat-stable antigen was indicated by cross-reactions involving over 160 cultures. This was confirmed by the absorption technic. The antigens of Cl. botulinum appear to be specific. Some cross-reaction was found with Clostridium sporogenes and Cl. parabotulinum, but the absorption technic was believed to provide a means of separation of these two species.

FROM THE AUTHOR'S SUMMARY.

THE IMMUNOLOIC RESPONSE TO VACCINIA IN GUINEA PIGS. L. DIENES and H. L. NATERMAN, J. Infect. Dis. **60**:279, 1937.

In order to obtain information concerning the significance of the bacterial type of allergy it should be studied in acute diseases. Vaccinia in guinea pigs was found to be an acute disease in which typical allergy develops. The skin reactions both grossly and microscopically correspond to tuberculin reactions.

The lesions produced in the skin of guinea pigs begin to heal on the fourth day after infection. Immunity of the skin becomes noticeable later, on the sixth or seventh day. Antibodies were not found before the twelfth day.

The development of allergy and the healing of the lesions before the development of general immunity indicate that healing is the result of an active process of immunity.

In rabbits the development of the tuberculin type of sensitiveness was not observed.

The possible participation of the dermal epithelium in the process of immunity and the similarities and differences of the process of disease in man and in guinea pigs have been pointed out.

FROM AUTHORS' SUMMARY.

PURIFICATION OF THE VI TYPE STRAINS OF BACILLUS TYPHOSUS. L. DETRE, J. Infect. Dis. **60**:319, 1937.

A new method is described for demonstrating the presence of Vi antibodies by the use of pure Vi suspensions of bacteria grown at 37 C. and freed from H antigens by consecutive washings. Such suspensions are not agglutinated by O or H antiserum but are easily agglutinated by Vi antiserum. Suspensions in salt sclution of living Vi bacilli grown at 37 C. retain their original qualities for three or four weeks if kept in the icebox. Formaldehyde is a good preservative.

POTENCY OF TYPHOID AND PARATYPHOID VACCINES WHEN FRESHLY PREPARED AND AFTER STORAGE. L. MISHULOW, I. MOWRY and A. K. STOCKER, J. Infect. Dis. **60**:356, 1937.

Potent typhoid and paratyphoid vaccines may be stored for several years without demonstrable loss of potency.

FROM AUTHORS' CONCLUSIONS.

LESIONS AND HISTOLOGIC CHANGES PRODUCED BY THE TOXIN OF VIBRION SEPTIQUE IN ANIMALS. J. G. PASTERNACK and I. A. BENGSTON, Bulletin 168, National Institute of Health Bulletin, 1936.

Intravenous injection of the toxin of vibrion septique (Clostridium œdematis-maligni) into rabbits, mice, guinea-pigs and pigeons consistently resulted in definite lesions. The myocardium showed hyaline as well as hydropic and colloid droplet degeneration, with areas of advanced necrosis and heavy infiltrations of degenerating and fragmenting polymorphonuclear leukocytes. The interstitial spaces of the heart were filled with red blood cells, and the capillaries were engorged with conglutinated cells and hyaline thrombi. The Purkinje cells of the conduction tissue were pale, swollen and hyalinized. The kidneys were surrounded with hemorrhagic extravasations and displayed red hemorrhagic and yellow necrotic mottling of the cortex with prominent glomerular blood cysts. Hydropic and

colloid droplet degeneration was present in the convoluted tubules and ascending limbs of Henle's loops. Hyaline fibrinoid and nonfibrinoid casts predominated. The glomeruli, necks and proximal convoluted tubules were filled with blood cells. Diffuse or focal tubular necrosis developed early. Hyaline and fibrin thrombi were frequently seen in segments of glomerular tufts. In severe cases there was extensive necrosis in stroma, vessels and parenchyma. The medulla exhibited vascular engorgement and hemorrhages. Only occasionally did foci of coagulation necrosis occur in the liver, irregularly disposed. Phagocytosis of hemosiderin by the Kupffer cells was marked. Distended with blood, the spleen rarely showed hemorrhagic infarcts, but necrosis in the malpighian corpuscles and thrombosis in the pulp were frequent. Phagocytosis of blood pigment was especially marked. Congestion, edema, hemorrhages, capillary hyaline thrombi and infiltration by polymorphonuclears were observed in the bone marrow, adrenals, brain, cord and thymus as well. Zenker's necrosis was found in the voluntary muscles.

FREDERICK STENN.

ANTIGENIC COMPOSITION AND VIRULENCE OF BACTERIUM TYPHOSUM GROWN ON A CHEMICALLY DEFINED MEDIUM. G. P. GLADSTONE, Brit. J. Exper. Path. **18**:67, 1937.

The presence of Vi antigen in strains of Bacterium typhosum is independent of the source of nitrogen used for growth, even when this is limited to ammonia only. A relation between the presence of Vi antigen in the cell and a source of carbon and energy has been established. Growing in the presence of dextrose, the organism retains its Vi antigen and is not agglutinated by a pure O antiserum. It remains virulent. Replacement of dextrose by lactate or by other 3-carbon or 4-carbon compounds leads to appearance of O-agglutinable forms which have lost their Vi antigen. It is suggested that the dextrose molecule is easily utilized by the organism for the synthesis of Vi antigen; the lactate molecule can be used only with difficulty.

FROM THE AUTHOR'S CONCLUSIONS.

THE SALT OPTIMUM IN ANTIBODY-ANTIGEN REACTIONS. J. T. DUNCAN, Brit. J. Exper. Path. **18**:108, 1937.

The antibody, antigen and salt optima giving the highest velocity of agglutination have been interpreted as follows: The antibody optimum determines the most effective antibody sensitization of the bacteria, and in the O forms of agglutination studied, it resulted in that antibody-antigen compound which had the highest cohesive properties, as judged by the resistance of the flocculi to dispersion by mechanical force. It may be said, therefore, to affect the quality (firmness) of agglutination. The antigen optimum depends on the highest concentration of adequately sensitized bacteria, and its effect is chiefly on the stage of particulation. The salt optimum determines the maximum combination of antibody with antigen and thus influences the quantity of agglutination measured by the highest effective serum titer. Antibody-antigen combination reaches its maximum at the salt optimum, and it diminishes progressively as the salt concentration deviates in either direction away from this optimum. The salt effects on the antibody-antigen reaction are reversible, so that dissociation of antibody from sensitized bacteria occurs when the salt concentration is altered from that at which they were sensitized to one more remote, in either direction, from the salt optimum; and additional antibody can be absorbed by the sensitized organisms if the alteration brings the salt concentration nearer to the optimum. The optimal salt concentration for the H form of agglutination is relatively low, and at high concentrations of the serum it tends to fall still lower as the suspension-serum ratio falls. The optimal salt concentration for the O form of agglutination is high in the presence of a high serum-suspension ratio, and it falls progressively with the fall in this ratio. A method of obtaining relatively pure H or O antibody by dissociation from sensitized bacteria is suggested.

FROM THE AUTHOR'S SUMMARY.

THE NERVOUS SYSTEM IN IMMUNITY. V. ZERNOFF, Ann. Inst. Pasteur **58**:212, 1937.

"In résumé, in accordance with the theory of Métalnikov, we have been able to prove once more the possibility of producing hemolysins under the influence of conditional reflexes. Thus, we have obtained an increase in the hemolytic titer of the blood without injections of antigen in ten of fifteen guinea-pigs. From fourteen to sixty injections of antigen associated with conditional excitations are necessary for the formation of conditional reflexes of guinea-pigs. Of ten animals which presented an increase in hemolytic titer under the influence of conditional reflexes, six formed these reflexes after the first series of injections of antigen, two after the second, and two after the third. The five control guinea-pigs presented no increase in titer of hemolysins after the second removal of blood."

AUTHOR'S RÉSUMÉ.

Technical

THE FRIEDMAN TEST FOR PREGNANCY. G. L. KELLY and E. B. WOODS, J. A. M. A. **108**:615, 1937.

The presence of one or more ruptured follicles in either ovary or in both ovaries in this test constitutes a positive reaction. The presence of several unruptured hemorrhagic follicles in both ovaries (usually two or more in each ovary) is also a positive reaction. Unruptured hemorrhagic follicles are red; black follicles of all sizes have no significance and must be disregarded. (Corpora lutea visible to the naked eye do not develop within forty-eight hours, nor can they be seen with low power binocular microscope. Only high magnification of histologic sections would show them.)

In many cases use of the low power binocular microscope is necessary in order to reach a correct decision and will prevent repetition of the test. For recognizing ruptured follicles use of this microscope should be a routine.

The common modification of the Friedman test is satisfactory, though the original Friedman technic gives better quantitative results. One injection of 10 cc. of urine with examination in thirty-six hours is not nearly so successful as the double injection, and better results would be obtained with two injections and examination in forty-eight hours.

Rabbits weighing more than 3 pounds (1,306 Gm.) will give better results than those weighing less, and 3 pounds is a safe minimum.

It is not necessary to acidify the urine, though it is better to do so, as the hormone present is more active in an acid medium. Urines do not kill test animals because of alkalinity.

It is safer not to use urines with a specific gravity of less than 1.008. If it is necessary to use such urines, the quantity injected in each instance should be increased at least 50 per cent.

FROM THE AUTHORS' SUMMARY.

A NEW APPARATUS FOR PROCURING STERNAL BONE MARROW. R. B. H. GRADWOHL, J. A. M. A. **108**:803, 1937.

For sternal punctures Gradwohl devised an apparatus consisting of a 10 cc. Luer Lok syringe, a cannula and a connector with a rubber tubing joint, an instrument for puncturing sternal bone marrow, with a guard which may be adjusted to fit the depth of the puncture, and an obturator for the latter. After the instrument has penetrated the sternum the obturator is removed, the connector and syringe are attached and suction is applied. Only from 0.2 to 0.3 cc. of bone marrow need be removed. Touch preparations are stained for thirty minutes with dilute Giemsa stain, made by adding a drop of Giemsa stain to 1 cc. of neutral distilled water. The slide is then washed with neutral distilled water, dried in the air and examined.

FREDERICK STENN.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

ESMOND R. LONG, *President*

Regular Meeting, Nov. 11, 1937

HERBERT L. RATCLIFFE, *Secretary*

EPIDEMIC ENCEPHALITIS (Gross Lecture). RALPH S. MUCKENFUSS, Director, Bureau of Laboratories, Department of Health, New York.

"Epidemic encephalitis" is the term in common use for a number of etiologically distinct diseases that involve the central nervous system. Encephalitis lethargica, described by von Economo, apparently made its appearance in this country about 1918, since which time it has been endemic. Of unknown etiology, this disease usually has a mild onset, after which there may be complete recovery, gradual progression until death ensues or alternating remission and exacerbation of symptoms.

Type B encephalitis of Japan appeared in epidemic form in 1924 and 1927. This disease occurs in summer epidemics, affects older persons, is accompanied by a rather high mortality and does not so commonly leave residua as the disease described by von Economo.

St. Louis encephalitis, first recognized as a disease entity in 1933, is similar in all essential characteristics to type B encephalitis of Japan but is somewhat milder. The etiologic agent is a filtrable virus, readily transmissible to mice and transmissible to rhesus monkeys, although it apparently cannot be maintained in this animal. The etiologic agent of the Japanese type B encephalitis was discovered in 1935 and is a filtrable virus capable of affecting mice and rhesus monkeys. This virus seems closely related to that causing St. Louis encephalitis but can be differentiated by proper laboratory methods.

ESMOND R. LONG, *President*

Regular Meeting, Dec. 9, 1937

HERBERT L. RATCLIFFE, *Secretary*

A REVIEW OF MALIGNANT TUMORS OF THE THYROID. LAWRENCE W. SMITH.

This presentation is a review of 146 malignant tumors of the thyroid.

The 146 tumors constitute the pooled material studied over a period of twelve years. Of all the specimens obtained in surgical operations on thyroids, the malignant tumors represent 2.1 per cent, or approximately one half of the total number of tumors of thyroids seen during this period, which total 4.3 per cent of all the specimens obtained in operations on thyroids. In the series of patients represented there was an approximate ratio of 6 females to 1 male. The ages ranged from 18 to 72 years, the average figure at the peak of incidence being 51.2 years.

Of the 146 cases, there was a history of preexisting adenoma in 136 (93.2 per cent), and in only 10 (6.8 per cent) of the cases could no such history be elicited. Of these 10 cases, 2 were instances of epidermoid carcinoma; 1 was a case of diffuse adenocarcinoma, in which the point of origin could not be determined, and

7 were instances of the small round cell type of malignant growth, 5 of which, I believe, represented a definite diffuse type of small cell carcinoma and the remaining 2 possibly a lymphosarcomatous variety.

I have attempted a classification in conformity with my original method of classification as published in 1929 from the Lahey Clinic. By this method all cases readily fall into six groups, and were I to stretch a point, two of the six groups might be considered as subgroups in one of the others.

Type 1: Papillary Type.—This is observed in approximately 30 per cent of the cases, the number in this series being 42. I believe this type to have its origin in a lateral thyroid anlage. It tends to occur in younger persons, although all ages are represented in this series. It has a very low degree of radiosensitivity. With adequate surgical treatment, recovery can be expected in as high as 70 per cent of the cases. Otherwise recurrence is the rule.

Type 2: Fetal Adenomatous Type.—This type is found in approximately 45 per cent of the cases, the number in this series being 68. These, I believe, have their origin in a mesial anlage. There is a wide distribution with respect to age, the great majority of the cases, however, falling in the period between 35 and 50 years. The evidence of malignancy is almost entirely dependent on evidence of invasion of blood vessels, although in the more marked cases invasion of the capsule and of adjacent tissue likewise occurs. This type is extremely radioresistant. Fortunately, of the cases showing invasion of blood vessels, approximately only a third show metastasis or recurrence after the growth has been adequately removed surgically. Operative procedures should give cures in from 40 to 50 per cent of the cases, as estimated by this series.

Type 3: Epidermoid Type.—This type occurs in approximately from 1 to 2 per cent of the cases; in this series it was noted in 2 cases. It usually arises from remnants of the thyroglossal duct, but, as has been recently shown, it may have an adenomatous origin, with metaplasia of the cells.

Type 4: So-Called Giant Cell (Undifferentiated Cell) Type.—This type is represented in approximately 10 per cent of the cases; in this series, in 16. It may occur in two forms, made up of (*a*) large polyhedral cells or (*b*) spindle cells. In every instance in this series it has arisen from a preexisting adenoma, and for that reason it might be considered as a subgroup of type 2. The incidence as to age shows that it occurs almost entirely between the ages of 50 and 65. The radiosensitivity varies somewhat, the growth in certain cases responding temporarily but subsequently showing greater malignancy. The usual outcome is death in from six to eighteen months. In this series, all but 3 patients died within eight months.

Type 5: Small Cell Type.—This type embraces approximately 10 per cent of the cases, the number in this series being 18. It also occurs in two forms: (*a*) a compact and (*b*) a diffuse form. The compact form also apparently arises from preexisting adenoma, but the diffuse form is usually so diffuse that it is difficult to determine its point of origin. The incidence with respect to age is similar to that of the giant cell tumor—being usually found in persons over 50. The small cell tumor is rapidly fatal; in the great majority of cases death occurs within six months after the diagnosis has been made. In all but 2 of the cases in this series the patients died within eighteen months. One of those excepted is still alive at the end of five years. This type occasions considerable argument as to its epithelial or mesothelial origin. The usual history is of a nodule which suddenly increases rapidly in size and is completely radioresistant. The involved region shows a diffuse infiltration of small round cells, difficult to differentiate from lymphocytes but with here and there sufficient suggestion of acinar formation to make one believe that they are of epithelial origin. In this group there have been 2 exceptions to the general rule, and I feel that these might be considered as lymphosarcoma because of their radiosensitivity and the fact that one of the patients is alive five years after operation and that the other lived for thirty-two months.

Type 6: Sarcoma of the Thyroid.—In reviewing the literature it will be noted that historically tumors of the thyroid were almost equally divided between carcinoma and sarcoma, but that in more recent studies the question has been raised, notably by Ewing, whether sarcoma of the thyroid has ever occurred. In my series the only cases in which any doubt has been felt have been 2 in the last group under discussion. In several others there has been possible sarcomatous degeneration of the stroma, but this I believe was only incidental to the basic carcinoma. One case reported by Zeckwer seems to fulfil the requirements for fibrosarcoma.

CELL POTENCIES AND THE RELATION BETWEEN A FEW INTRACELLULAR CHEMICAL COMPOUNDS AND THE BIOLOGY OF GROWTH. STANLEY P. REIMANN.

The potency of a cell is always greater than the realization of that potency. A completely differentiated cell can divide no longer.

Therefore tumors must arise from cells that are incompletely differentiated. Therefore the potency of these cells is greater than its realization. This is of prime importance in a consideration of the genesis of tumors.

Potency can be regarded from the chemical point of view as the presence within cells of chemical compounds and groups capable of rearrangement and union; that is, differentiation is chemical reorganization.

Various chemical compounds and groups have been identified as being present within cells. The primary problem of the Lankenau Hospital Research Institute is concerned with the chemical factors concerned in growth and development. Therefore the question was asked years ago, What part do the various intracellular chemical constituents play in growth and development? Numerous animals and plants have been used as biologic test mediums. For the last five years Hammett has used the marine hydroid Obelia geniculata, in which it is possible to divide growth and developmental activities into at least thirty separate phases. It has been found that

(*a*) Aminoacetic acid acts specifically to accelerate regeneration.

(*b*) d-alanine has no apparent effect on the growth of the animal other than that which can be attributed to its specific dynamic action.

(*c*) l-phenylalanine may, as a potential source of tyrosine, act in some small degree as a forwarder of differentiation.

(*d*) d-glutamic acid hastens differentiation and organization.

(*e*) l-tryptophan retards catabolism and thus may act as an indirect stimulus to general growth.

(*f*) Histidine may act similarly through its sustaining effect on metabolic expenditure.

(*g*) Uracil favors recurrent growth and retards differentiation, regeneration and metaplasia.

(*h*) d-arginine has an effect similar to that of uracil, which suggests the latter as a natural precursor of the former.

(*i*) d-ribose has no specific effect on any of the developmental growth activities, but acts as a source of material yielding energy.

(*j*) Allantoin may favor growth in general but it has no specific influence on the increase in cell number.

(*k*) Cysteine favors proliferation.

(*l*) Methionine favors proliferation.

(*m*) l-tryosine is a determinative participant in some process specific to differentiation.

(*n*) Cytosine accelerates differentiation and retards initiation.

(*o*) Thymine is of significance in both proliferation and differentiation.

(*p*) l-aspartic acid furthers differentiation.

SPONTANEOUS AND CHEMICALLY INDUCED TUMORS IN MICE. H. B. ANDERVONT, Biologist, Office of Cancer Investigations, United States Public Health Service, Harvard Medical School, Boston (by invitation).

Spontaneous Tumors.—By brother to sister mating for twenty or more generations, certain strains of mice have been obtained in which most of the breeding females acquire spontaneous carcinoma of the mammary glands while in other strains there is a low incidence of such growths in breeding females. It has been found that the susceptibility to the development of these growths is influenced through the maternal line only, which means that the influence is extrachromosomal. Recent investigations suggest that mother's milk may play an important role in the appearance of mammary cancer in mice.

Strains of mice have also been developed with a high incidence of spontaneous pulmonary tumors in which the susceptibility to the development of these growths is inherited as a dominant characteristic and is in accordance with genetic principles.

The discovery that the underlying mechanism of inheritance is different for these two types of tumors in mice tends to complicate any studies relating to the inheritance of the susceptibility to tumor in other species.

Chemically Induced Tumors.—From the diverse chemical nature of pure compounds capable of producing tumors in mice, it is clear that there is no possibility of arriving at any generalization regarding the molecular structure necessary for cancer-incitement. One compound has been obtained which is a degradation product of desoxycholic acid and of cholic acid, which are constituents of the bile. This suggests that some malignant growths may owe their origin to the production in nature of carcinogenic substances from bile acid, sterols or gonadotropic substances.

Certain carcinogenic hydrocarbons produce tumors in all strains of inbred mice, but there is a striking difference in the average time of appearance of induced tumors in the different strains. This pronounced variation in susceptibility is further evidence of differences in the genetic constitution of various strains of inbred mice. There is no correlation between the susceptibility of mice to the development of spontaneous mammary growths and their susceptibility to the development of tumors induced at the sites of injection of carcinogenic hydrocarbons. Each strain of mice has its own degrees of susceptibility to spontaneous, induced and transplantable tumors. Thus far a strain of mice uniformly susceptible or resistant to tumor growth has not been found.

Carcinogenic hydrocarbons produce a wide variety of tumors when placed in contact with different tissues. This implies that a single causative agent may be responsible for different types of tumors in some species of animals. Some of the carcinogenic hydrocarbons, when injected subcutaneously into certain strains of mice, induce tumors not only at the sites of injection but also in the lungs. Since, in these experiments, there is no known opportunity for the inhalation of the cancer-inducing compound, the question arises whether many pulmonary tumors in other species owe their origin to the inhalation of contaminated air.

Book Reviews

Diseases of the Blood and Atlas of Hematology, with Clinical and Hematologic Descriptions of the Blood Diseases Including a Section on Technic and Terminology. By Roy R. Kracke, M.D., Professor of Bacteriology, Pathology and Laboratory Diagnosis, Emory University School of Medicine; Pathologist to the Emory University Hospital; Consultant in Hematology to the Grady Hospital and Eggleston Hospital for Children, Atlanta, Ga., and formerly Director of the Hematological Registry, American Society of Clinical Pathologists, and Hortense Elton Garver, M.S., Instructor in Laboratory Diagnosis, Emory University School of Medicine. Pp. 532, including 44 color plates and 17 other illustrations. Price, $15. Philadelphia, London and Montreal: J. B. Lippincott Company, 1937.

The book is divided into eight sections and forty-one chapters. The first section deals with terminology and definitions of hematologic terms. It attempts to introduce order into this badly confused branch of medicine. The terminologic anarchy is possibly at its worst in hematology, and any endeavor to correct it is to be commended. The suggestions of Kracke and Garver may not be acceptable to all; other systems of classification will be forthcoming, but it is hoped that eventually terms will become crystallized to mean the same objects to all. That Kracke and Garver have not escaped the usual tendency of creators of new systems to go a bit too far is evidenced by, among other things, the inclusion of the term "fragilocyte" among the descriptive morphologic terms relating to the red cells. That inclusion is not justified, because fragility is not one of the morphologic features of the red cells.

The six chapters of section 2 deal with the theories of origin and development and the morphology of blood cells. The arguments brought forward by the leading authorities are presented in an objective manner.

Section 3 takes up the morphology of normal blood and the conditions associated with an increase and with a decrease in the numbers of white blood cells.

The eleven chapters of section 4 are given to a very complete presentation of the anemias; then follow the leukemias in section 5 and the hemorrhagic diseases in section 6. Section 7 includes such miscellaneous topics as infectious mononucleosis, polycythemia, the bone marrow, malaria, the blood groups and the blood picture of normal laboratory animals. The sixty pages of the last section are devoted to hematologic technic.

James J. Clark, Francis P. Parker, Elizabeth Gambrell and R. P. Custer contributed chapters on the roentgenologic treatment of the leukemic states, on the blood groups and blood transfusion, on malaria and on the bone marrow, in the order named.

A brief but carefully selected list of important references is included at the end of each chapter. It is not complete, but it was undoubtedly not intended to be for a book of this type. A subject index of fourteen pages concludes the volume.

Those who treat patients with diseases of the blood as well as those who help to recognize these diseases by examining the blood have long felt the need of a book just like the one by Kracke and Garver. They were on the lookout for a monograph that would offer more than the brief chapter or chapters in the usual texts or systems of medicine, that would include acceptable reproductions of blood cells as they are actually seen with the microscope and that would present the modern treatment of diseases of the blood. That need is now fulfilled. This monograph will take care of the requirements of the practitioner, and it will go a long way to help the hematologic worker in the laboratory.

The color reproductions of blood cells deserve the highest praise. They are the work of the co-author, Hortense Garver. These color plates are among the best in this field. A careful study of them and comparison with cells on well stained smears under the microscope should help those who want to learn hematology and who realize that there is no royal road to knowledge. On the pages opposite the tables a schematic sketch with numbers under the corresponding cells facilitates identification. It is regrettable that they are not of the same size as the actual plates. That would have made it easier to localize the corresponding cells, and it would not have required more space than was available.

The subjects are treated thoroughly, clearly and without the dogmatism that is so common in hematologic literature. The style is lucid. The recent contributions to the subject are adequately treated, and due consideration is given to the limitations of knowledge.

Minor errors—for instance, in the formula for the calculation of the volume index (page 482) or in the presentation of the laws of heredity of the M and N factors (page 443)—and a few errors in printing can easily be corrected. Concerning the M and N factors it is stated "combinations (group M and the Group N child) or (group N man and group M child) cannot occur." It should be "combinations (group M parent, etc.) and (group N parent, etc.).

Books Received

SURGICAL PATHOLOGY OF THE DISEASES OF THE NECK. HERTZLER'S MONOGRAPHS ON SURGICAL PATHOLOGY. Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kansas, and Professor of Surgery, University of Kansas. Cloth. Price, $5. Pp. 237, with 206 illustrations. Philadelphia: J. B. Lippincott Company, 1937.

NEULAND IN DER HEILKUNDE. Dr. Henri Hirsch. Paper. Price, 3.20 Swiss francs. Pp. 87. Basel, Switzerland: S. Karger, 1937.

ORIGIN AND DEVELOPMENT OF MEDIASTINAL AND AORTIC THYROIDS AND THE PERIAORTIC FAT BODIES. A COMPARISON OF DOG, FOX, PIG, OPOSSUM, AND MAN. Otto Frederic Kampmeier, Ph.D., M.D., Professor of Anatomy and Head of the Department. Illinois Medical and Dental Monographs, vol. 2, no. 1. Cloth, Price, $2. Pp. 82, with 65 illustrations. Urbana: University of Illinois, 1937.

CUTANEOUS CANCER AND PRECANCER. A PRACTICAL MONOGRAPH. George M. Mackee, M.D., Professor of Clinical Dermatology and Syphilology and Director of Skin and Cancer Unit, New York Post-Graduate Medical School and Hospital, Columbia University, and Anthony C. Cipollaro, M.D., Associate in Dermatology, Skin and Cancer Unit, New York Post-Graduate Medical School and Hospital, Columbia University. Foreword by Francis Carter Wood, M.D. Cloth. Price, $3.75. Pp. 222, with 232 illustrations. New York: American Journal of Cancer, 1937.

INDEX MEDICO-PHARMACEUTIQUE. N. T. Deleanu, professeur à la Faculté de pharmacie de Bucarest, Réné Fabre, professeur à la Faculté de pharmacie de Paris, and L. Coniver, ancien assistant à l'Université de Bucarest. Cloth. Price, 150 francs. Pp. 756. Paris: Masson & Cie, 1937.

A THEORY OF CANCER AND MNEMOTHERAPY. R. Roosen, M.D., English translation revised by C. F. Marshall, M.D., F.R.C.S. Cloth. Pp. 75. London: Bailliere, Tindall & Cox, 1936.

OM 1:2:5:6-DIBENZANTHRACENSARKOMENES UTVIKLING. Einar Hval. Papers published by the Klaus Hanssens Fond. No. XIV. Paper. Pp. 141, with 69 illustrations. Oslo: Nationaltrykkeriet, 1937.